

Supporting Information

New structures, spectrometric quantification and Na⁺/K⁺ inhibitory properties from cardenolides of *Asclepias curassavica* seeds

Authors

Paola A. Rubiano-Buitrago^{1,2}, Shrikant Pradhan¹, Christian Paetz² and Hannah M. Rowland¹

Affiliations

1. Research Group Predators and toxic prey, Max Planck Institute for Chemical Ecology, Jena, Germany, pbuitrago@ice.mpg.de (PARB), hrowland@ice.mpg.de (HMR).
2. Research Group Biosynthesis/NMR, Max Planck Institute for Chemical Ecology, Jena, Germany, cpaetz@ice.mpg.de.

Correspondence: PRB pbuitrago@ice.mpg.de HMR hrowland@ice.mpg.de

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Figure S1: UV and MS chromatograms of *Asclepias curassavica* extract (compounds **1-10** above their correspondent peak)

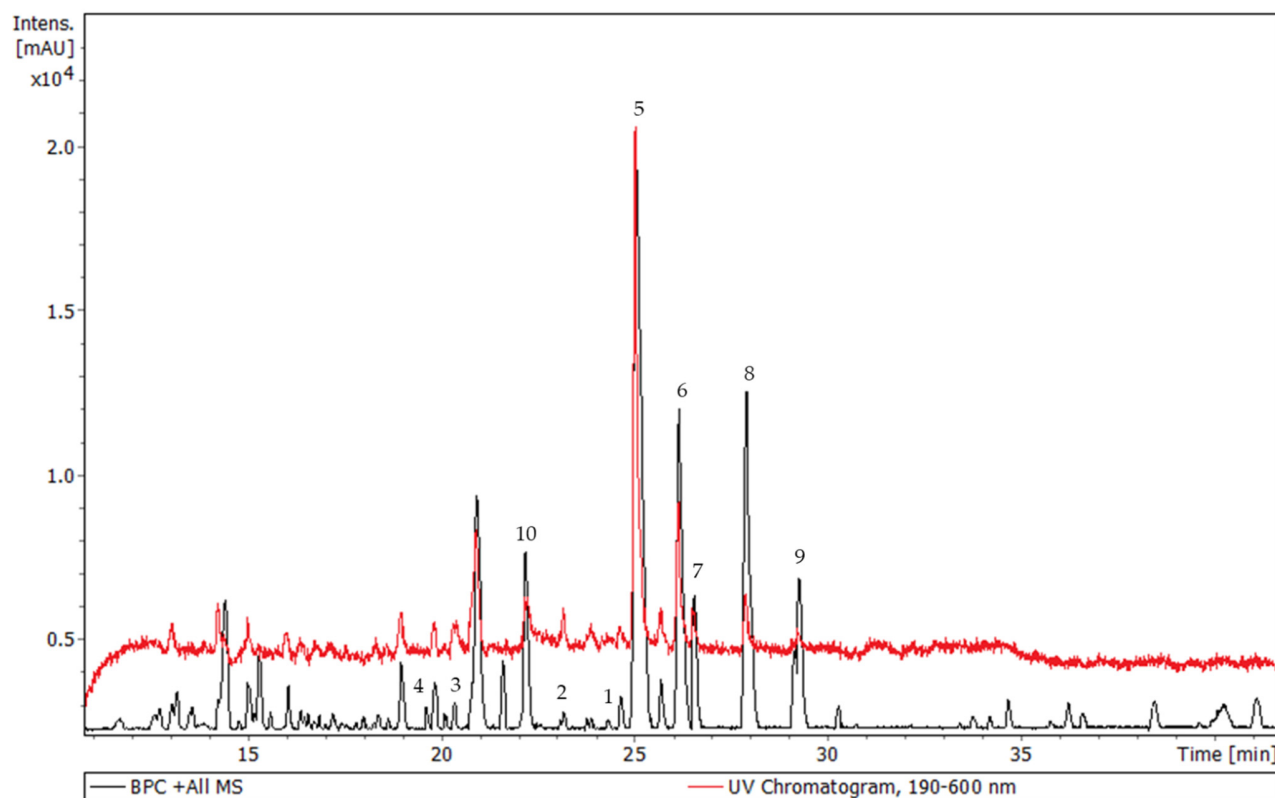


Figure S2: Calibration curves of compounds 1-10

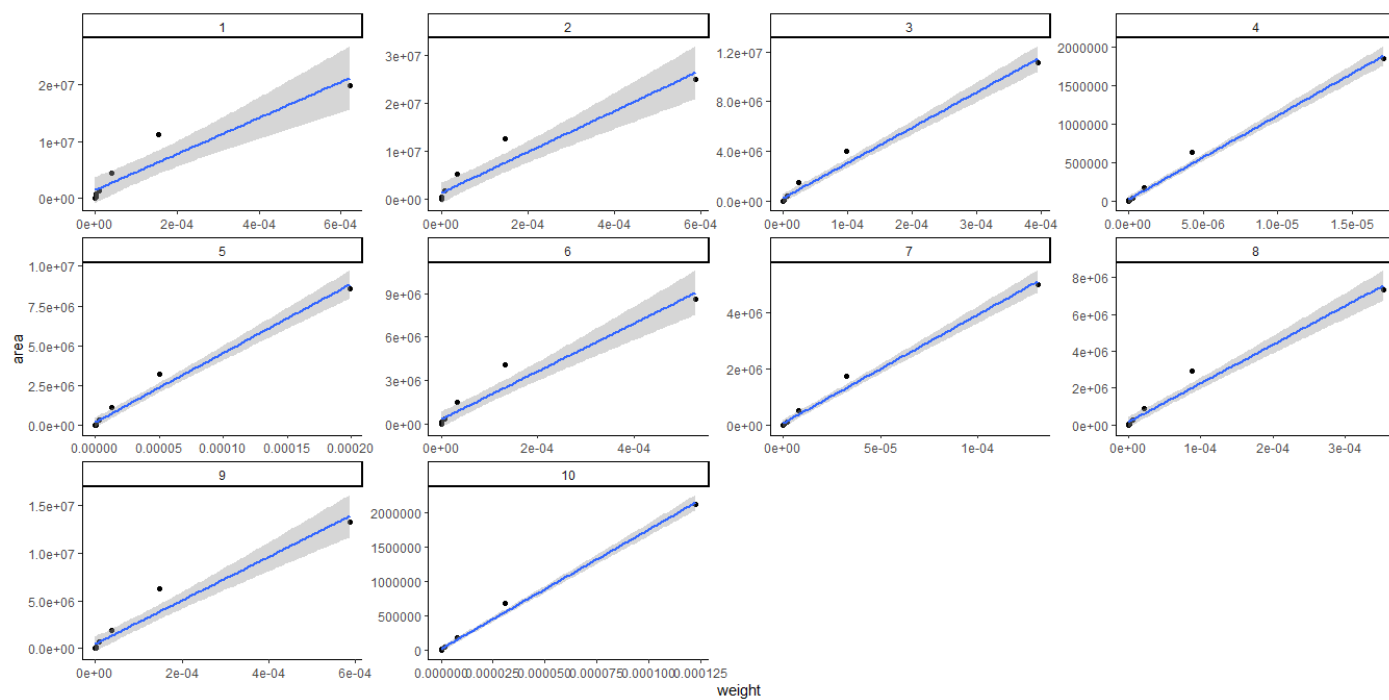


Table S1: ¹H NMR (500 MHz) and ¹³C (125 MHz) data of compounds 5-10 [δ in ppm, configuration, multiplicity (*J* in Hz)]

Position	5*		6*		7*		8**		9*		10*	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1	2.26 β , 0.92 α	30.8	2.39 bd (13.4) 1.06 bt (13.4)	30.1	2.46 β dd (12.6,4.3) 1.19 α t (12.6)	34.4	2.39 β dt (13.6,2.9) 1.08 bt (13.6)	30	2.31 β dt (13.1, 3.3) 0.78 td (13.1, 3.3)	32.5	2.37 β dd (12.6,4.4) 1.12 α t (12.6) 3.74 β ddd	34.3
2	1.44, 1.94	29.1	1.97, 1.24	29.8	3.90 β	69.1	1.98, 1.24	29.8	1.88, 1.53	30.7	(12.6,10.7, 4.4)	69
3	3.85 α	78.8	3.79	78.4	4.03 α t (3.0)	72	3.83 α	78.3	3.67 α	79.2	3.89 α td (10.7, 4.0)	72.1
4	1.41, 1.83	34	1.99, 1.21	35.4	1.78, 1.35 β	30.8	1.99 α , 1.2 β	35.7	1.76, 1.42	35.8	1.67, 1.24 q (12.6)	32.7
5	1.3 α	43.8	1.51	42.1	1.71	42.3	1.6 td (12.2, 3.1)	42.3	1.21	45.6	1.6 α	42.3
6	1.36, 1.36	26.7	1.94, 1.68 2.16 bd (12.3)	27.7	2.19, 1.77 1.98 β qd (13.1,3.1)	26.7	2.16, 1.35	26.7	1.31, 1.21	29.3	1.86 bdd (13.1, 3.3), 1.26	26.8
7	1.98, 1.18	27.1	1.32	26.8	1.39	26.8	1.95 β , 1.69	27.8	2.06, 1.13	28.6	2.08, 1.66	26.7
8	1.74 β	41.3	1.6 t (12.4)	42.3	1.65 β	41.6	1.53 β	42	1.77	42.8	1.53 β	41.6
9	1.12 α	49.2	1.41	47.8	1.58	47.5	1.44 α	47.7	1 α m	51.3	1.52 α	47.4
10		39.1		51.9		53.2		52.1		40.3		53.2
11	1.66, 1.44	22.5	1.71, 1.12	21.5	1.73 1.16 β	21.5	1.73, 1.13 β	21.5	1.62, 1.62	23.7	1.68, 1.09	21.8
12	1.44, 1.55 α	39.7	1.53, 1.44	38.7	1.56, 1.47, bt (13.6)	38.4	1.52, 1.44	38.9	1.48, 1.39	41.3	1.63, 1.57	39.6
13		49.2		49.4		49.4		49.5		50.9		48.9
14		86.3		85.7		85.6		85.8		86.4		85.4
15	2.20, 1.73	31.7	2.2, 1.71	31	2.16, 1.74	30.8	2.14, 1.71	31.1	2.14, 1.69 dd (11.5;9.5)	33.3	2.06, 2.06	39.1
16	2.22, 1.84	26.5	2.12, 1.83	26.5	2.21, 1.82	26.4	2.21, 1.83	26.5	2.16, 1.86	27.8	4.48 β td (8.2,4.1)	76.1
17	2.91 α	50.1	2.88	49.9	2.92 dd (9.5, 5.6)	49.7	2.9 α dd (9.5, 5.4)	50.2	2.82 α	52.1	2.62 α d (5.1)	59.2
18	0.95 s β	15.2	0.81 β s	15.1	0.83 β s	14.9	0.82 β s	15.1	0.92 β s 3.86 dd (11.7, 4.2)	16.2	0.71 β s	14.9
19	3.92, 3.77	60.1	10 s	214.9	10.09 β s	212.8	9.95 β s	214.9	3.73	60	9.96 s	212.9
20		178.9		178.7		178.4		178.5		176.9		175.4
21	5.06 dd (18.2, 1.3) 5.08 dd (18.2, 1.3)	75	5.06 bd (18.5) 5.01 bd (18.5)	75	5.07 dd (18.2, 1.3), 5.04 dd (18.2, 1.3)	75	5.08, 5.03	75.1	5.03, 4.90	75.2	4.92, 4.92	75.1
22	6.00 s	116.1	5.98 s	115.8	6.00 s	116	5.97 s	116	5.89 s	117.5	5.96 s	116.7
23		179.4		178.9		178.8		178.9		178.1		177.9
1'	4.87 α d (8.3)	97.9	4.82 α d (7.9)	97.8	4.65 β s	94.8	4.81 α d (8.2)	98	4.72 d (8.0)	99.5	4.53 β s	95.2
2'	3.46 β	69.7	3.4 β dd (7.7,1.8)	70		91.6	3.38 dd (8.2,3.1)	70.5	3.26 dd (7.9, 2.9)	72.6		91.8

3'	4.4 β t (2.8)	70.7	4.37 β bd	70.5	3.96 β dd (11.6, 4.5) 2.15 β bdd (12.0, 4.5)	81.5	4.13 α t (3.1)	71.2	4.01 α t (2.9)	73	3.68 β dd (12.1,5.0) 1.76 β ddd (12.6,4.8,1.3),	71.6
4'	3.53 β	82	3.49 β d (9.4) 3.9 α bdt	81.6	1.73 q (11.3, 12.0)	36.9	3.35 dd(9.8,3.1)	72.4	3.14 dd (9.5,2.7)	74.6	1.54 α	37.2
5'	3.94 α	68.2	(6.2, 6.2, 3.2)	68.4	3.84 β dd (11.3,5.9)	68.6	3.8 α m	69.4	3.71 α	70.4	3.72 β	68.9
6'	1.34 β d (6.2)	16.8	1.31 β d (6.2)	16.8	1.3 α d (5.9)	19.7	1.25 β d (5.9)	16.7	1.22 β d (6.3)	18	1.19 α d (5.7)	19.7
1"	4.6 α d (7.8)	104	4.58 α d (7.8)	104	4.66 α d (7.9)	104						
2"	3.35 β bt (8.3)	73.1	3.32 β bt (8.8)	73.2	3.39 bt (8.7)	73.3						
3"	3.5 α	75.7	3.43 α	75.6	3.53 α t (9.1)	75.5						
4"	3.46 β	75.7	3.42 β	69.4	3.44 m	69.3						
5"	3.46 α	75.8	3.48 α t (9.4)	75.6	3.46 α m	75.8						
6"	3.92, 3.77	60.1	3.89 bd (12.1) 3.75 dd (12.1,3.3)	60.2	3.92 bd (12.2) 3.76 dd (12.2, 5.1)	60.4						

*-in D₂O, **-in MeOD-d₃

Figure S3: HR-ESI-MS spectrum of compound 1.

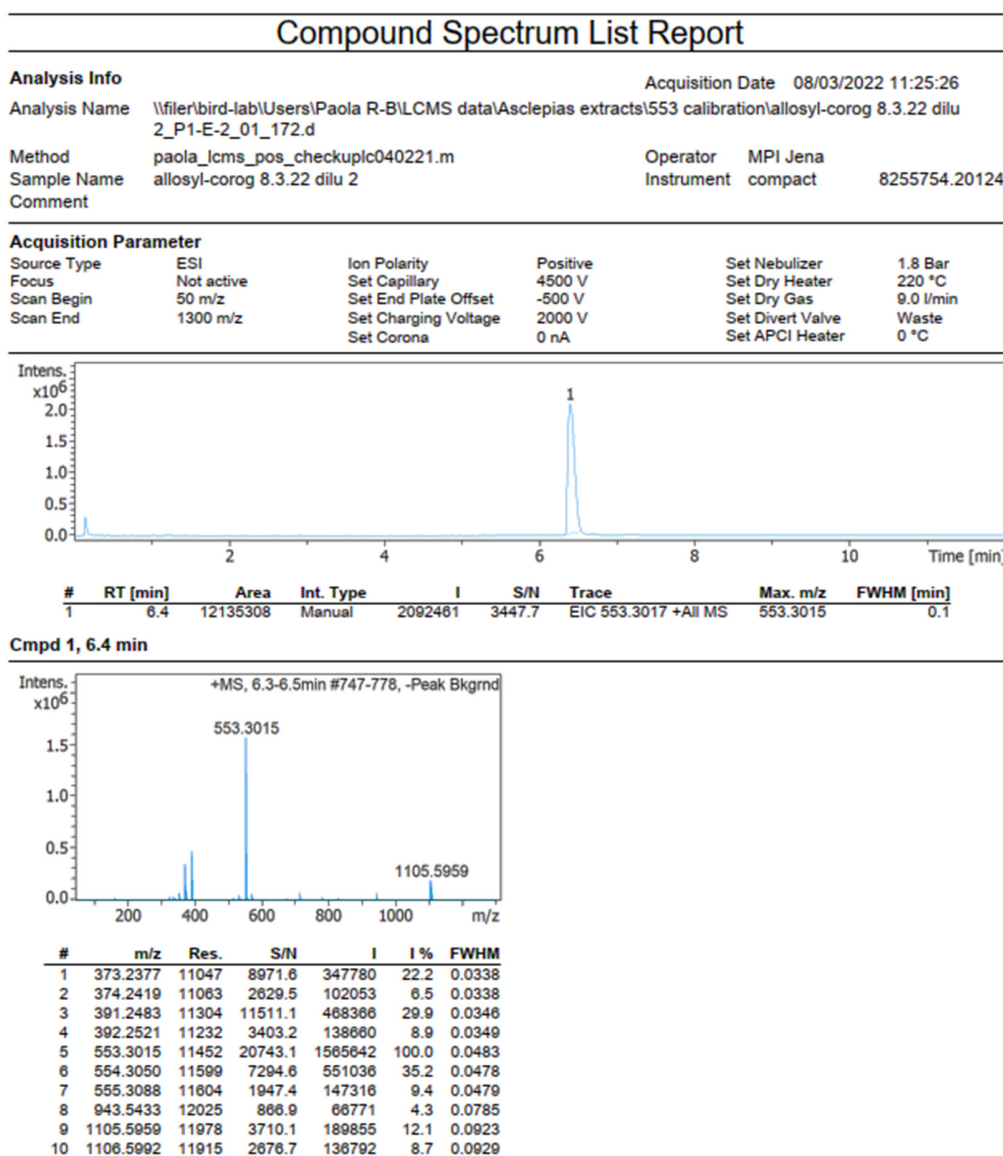


Figure S4: UV spectrum of compound **1**

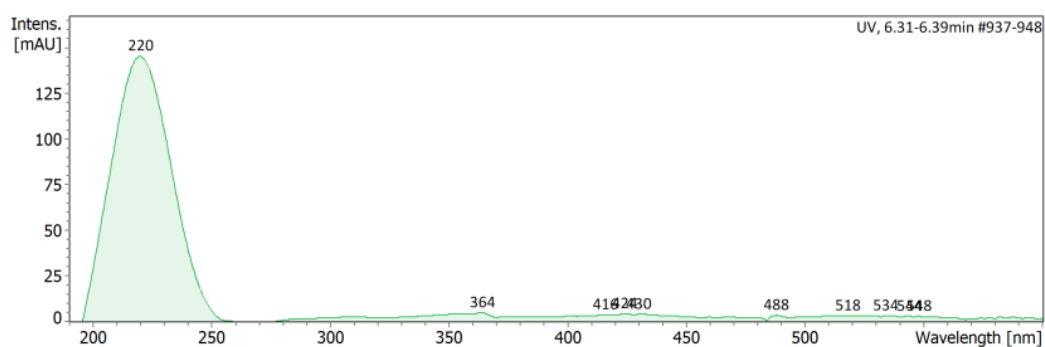
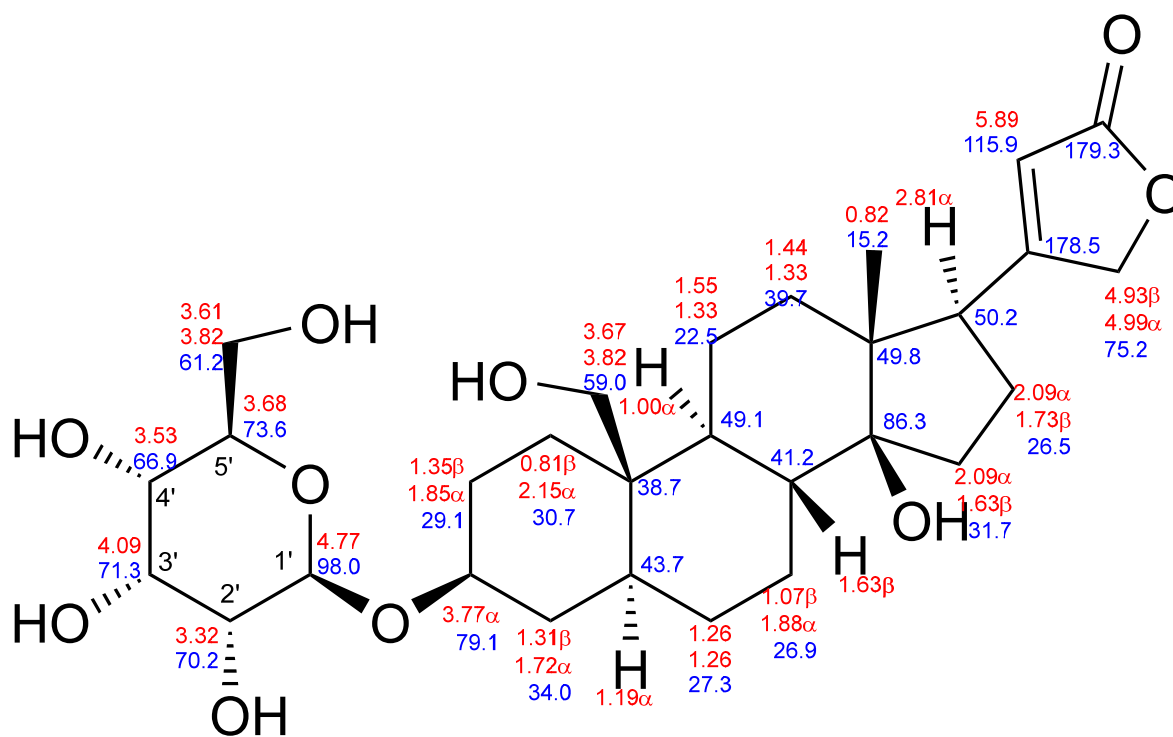


Figure S5: Compound **1**-NMR shift assignment



3β-(β-D-allopyranosyloxy)-14,19-dihydroxy-5α-card-20(22)-enolide

Figure S6. ¹H-NMR spectrum (500 MHz, D₂O) of compound **1**.

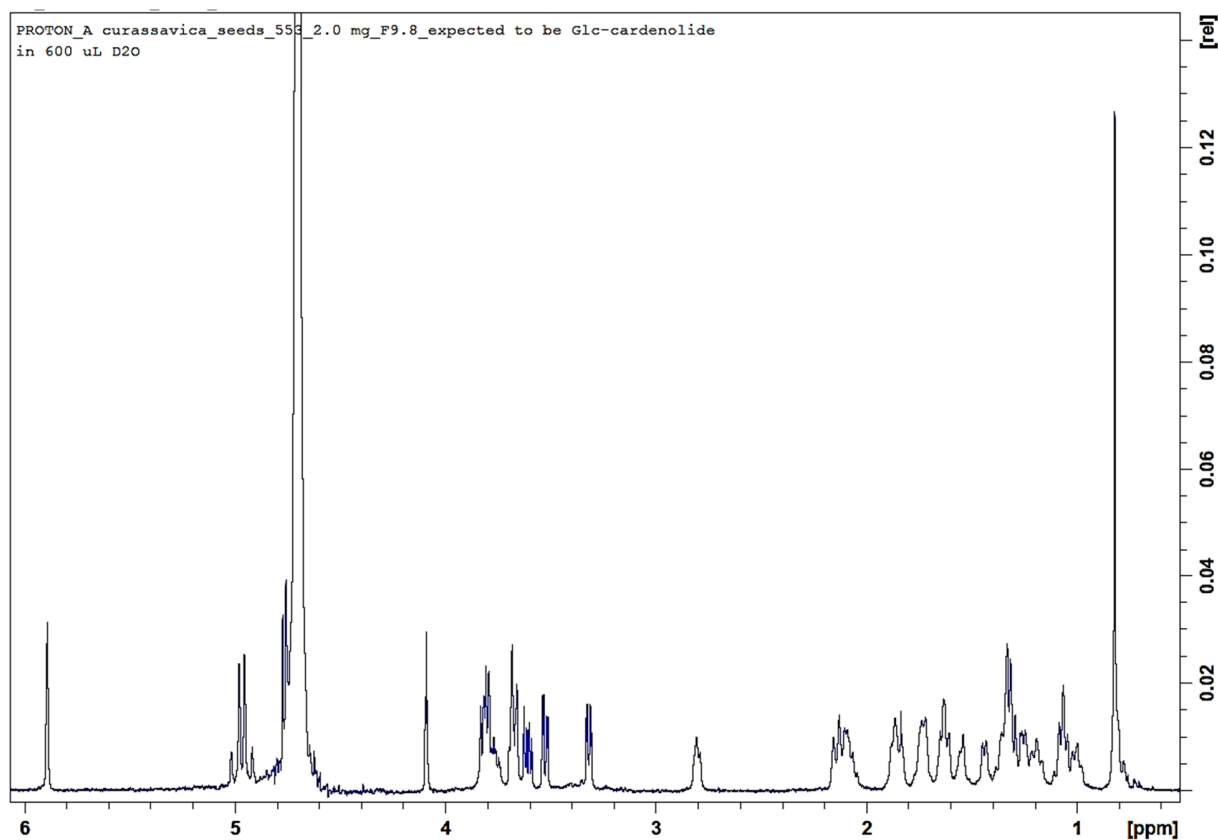


Figure S7. DEPTQ spectrum (125 MHz, D₂O) of compound 1.

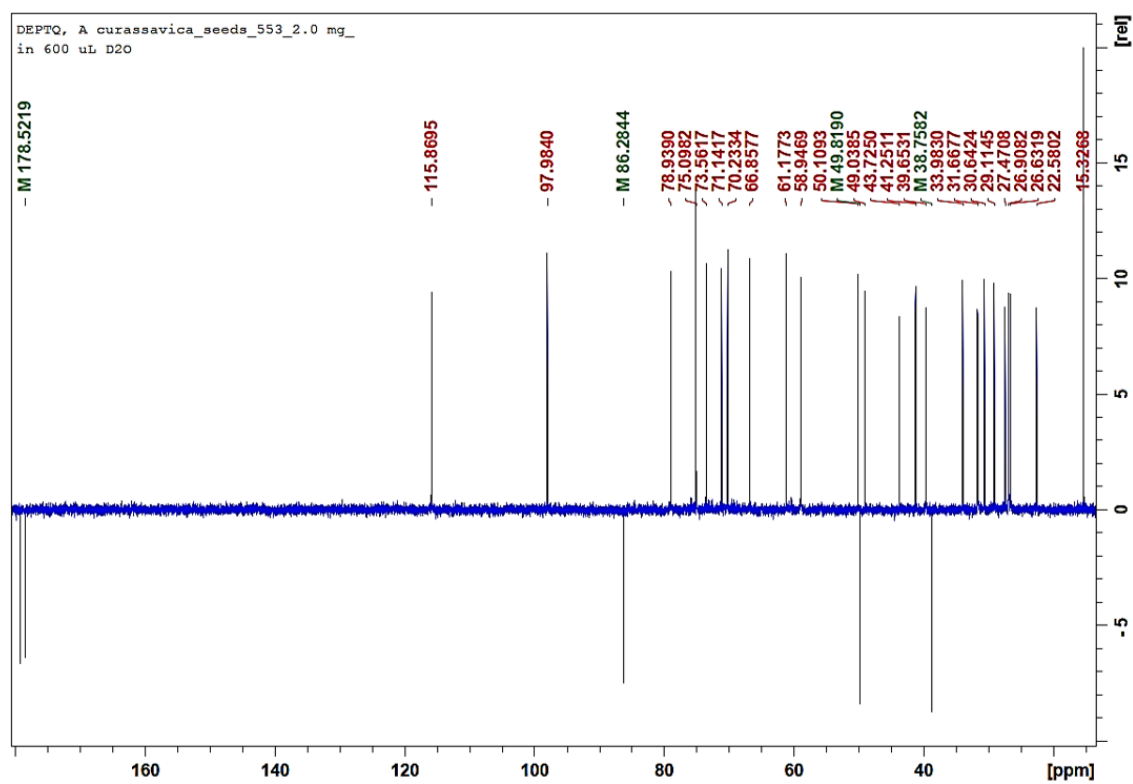


Figure S8. HSQC spectrum (500 MHz, D₂O) of compound 1.

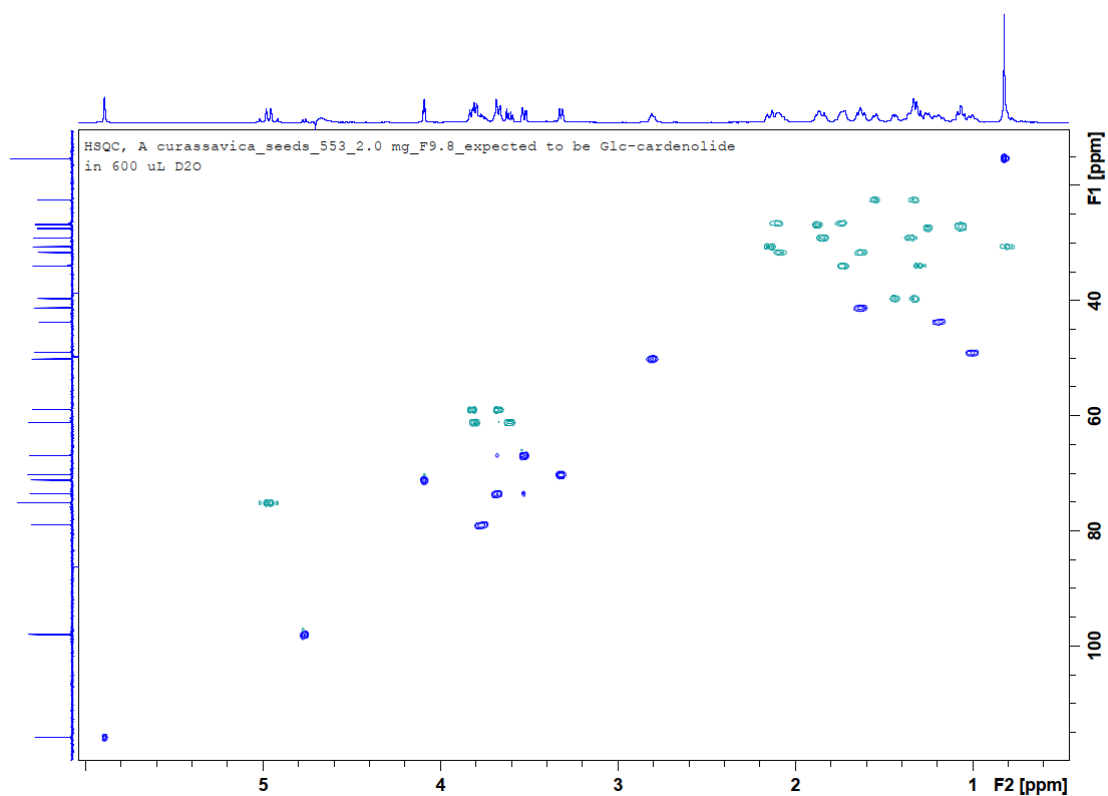


Figure S9. ^1H - ^1H COSY spectrum (500 MHz, D_2O) of compound **1**.

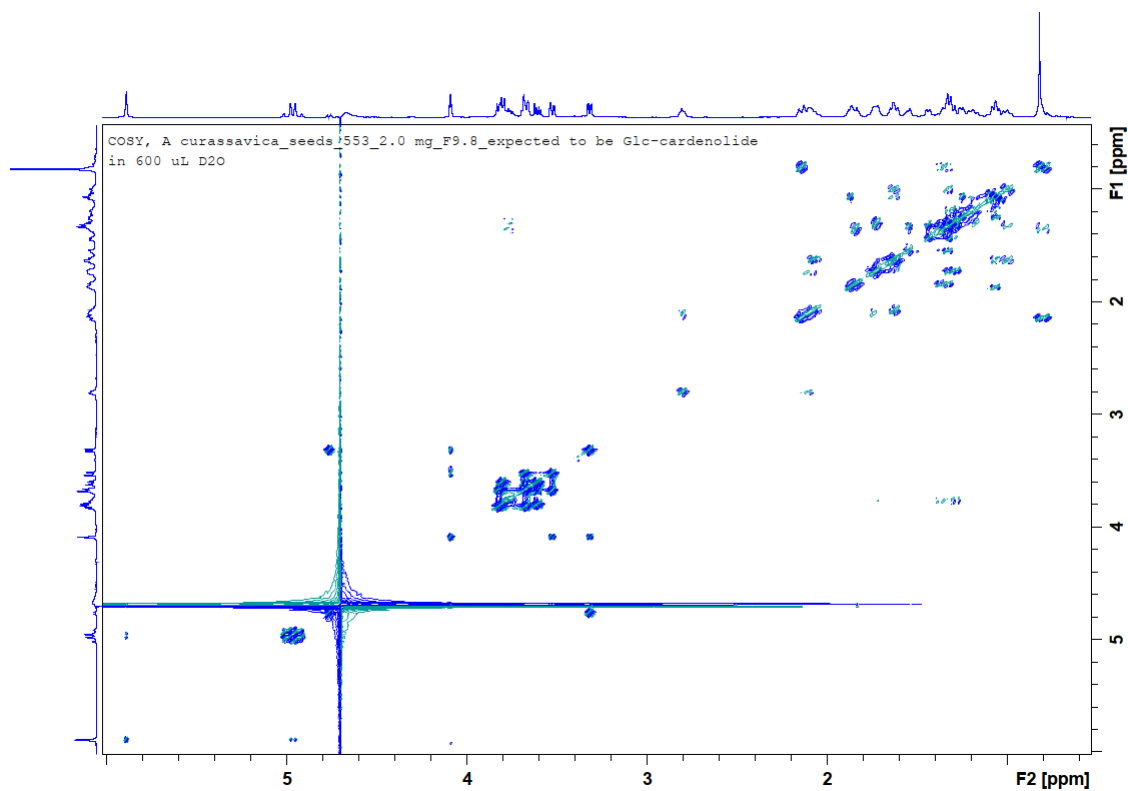


Figure S10. ^1H - ^{13}C HMBC spectrum (500 MHz, D_2O) of compound **1**.

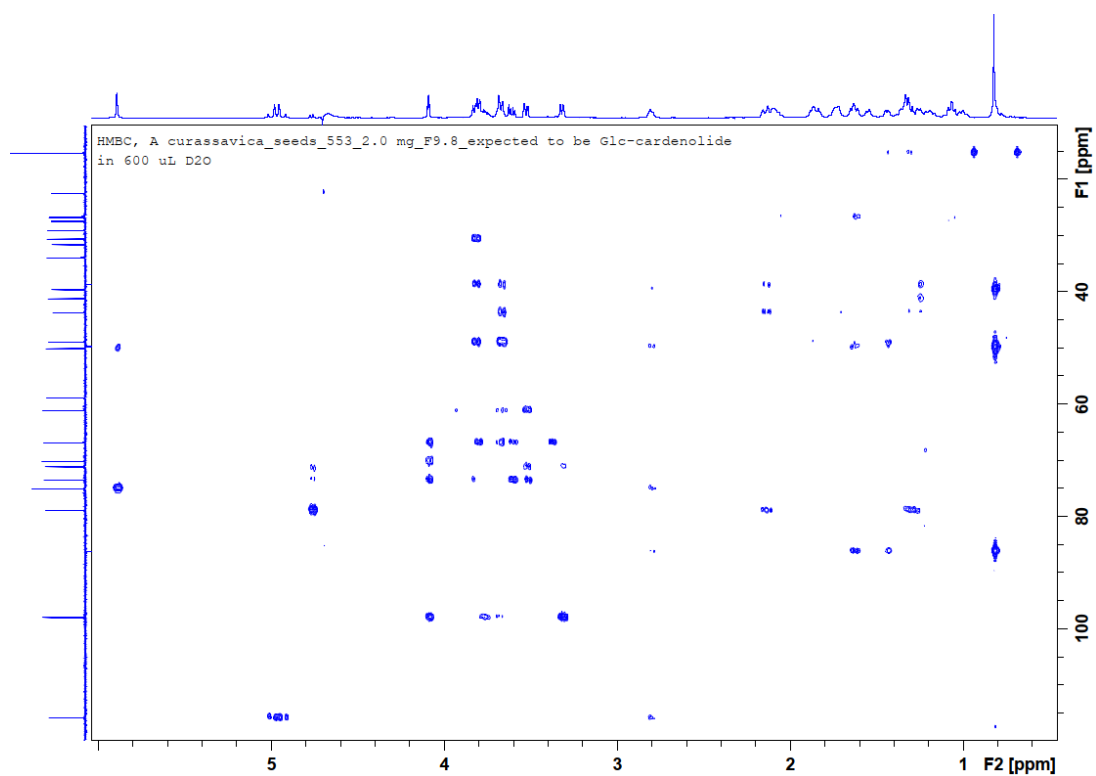


Figure S11. ROESY spectrum (500 MHz, D₂O) of compound **1**.

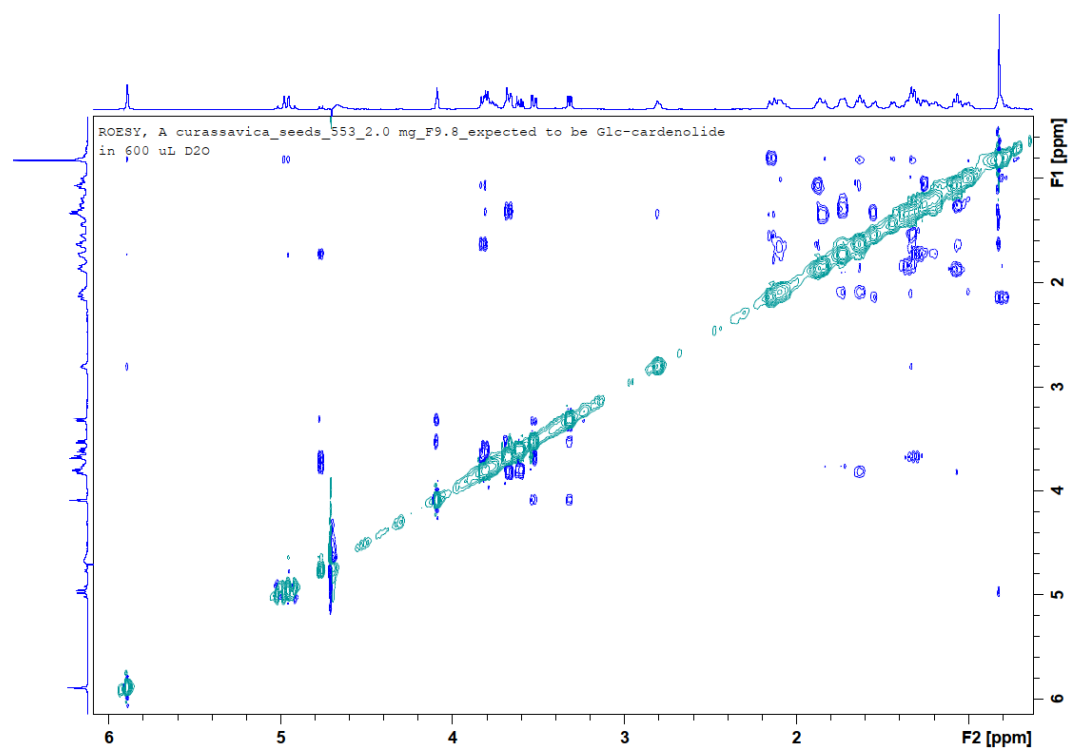


Figure S12. HSQC-TOCSY spectrum (500 MHz, D₂O) of compound **1**.

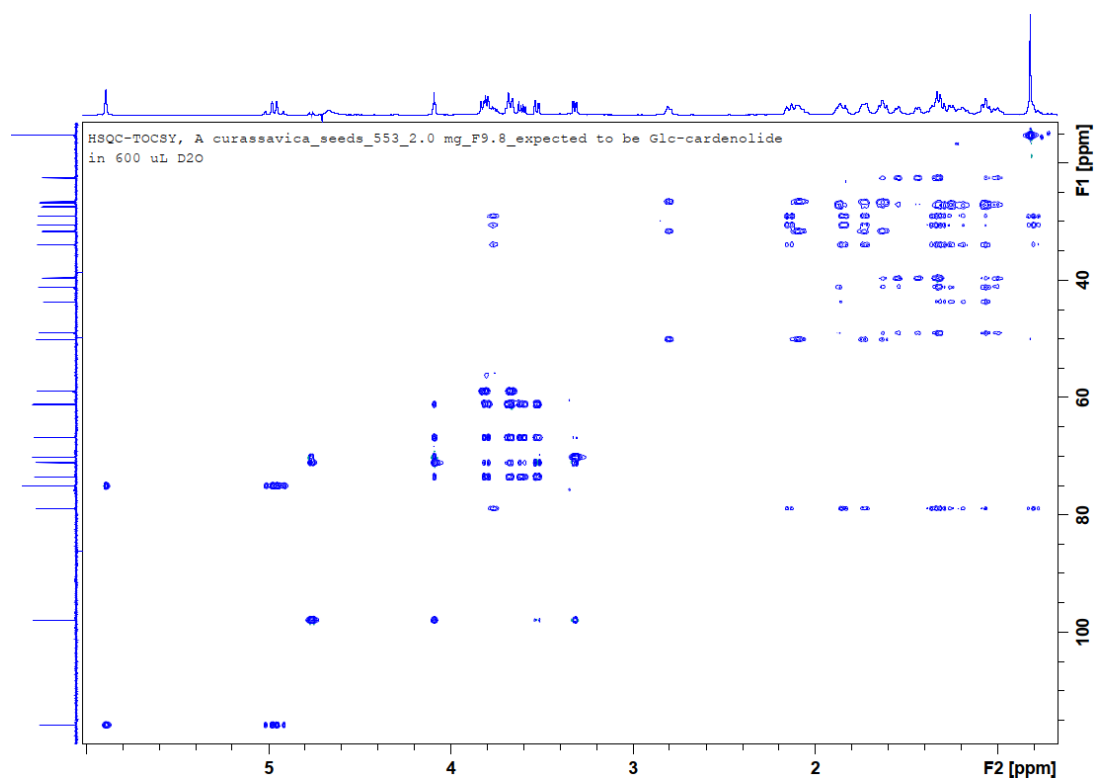


Figure S13. SELTOCSY spectrum (500 MHz, transmitter frequency at 3.609 ppm (H-6'), D₂O) of compound **1**.

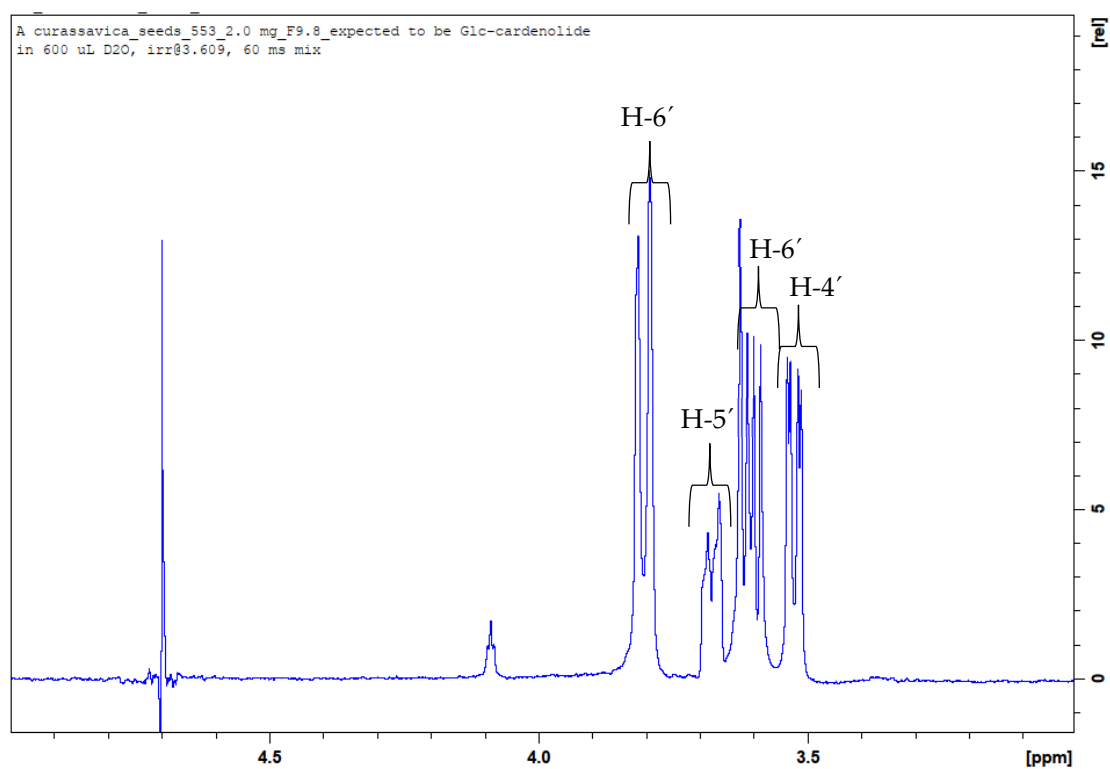


Figure S14. SELTOCSY spectrum (500 MHz, transmitter frequency at 3.765 ppm (H-3), D₂O) of compound **1**.

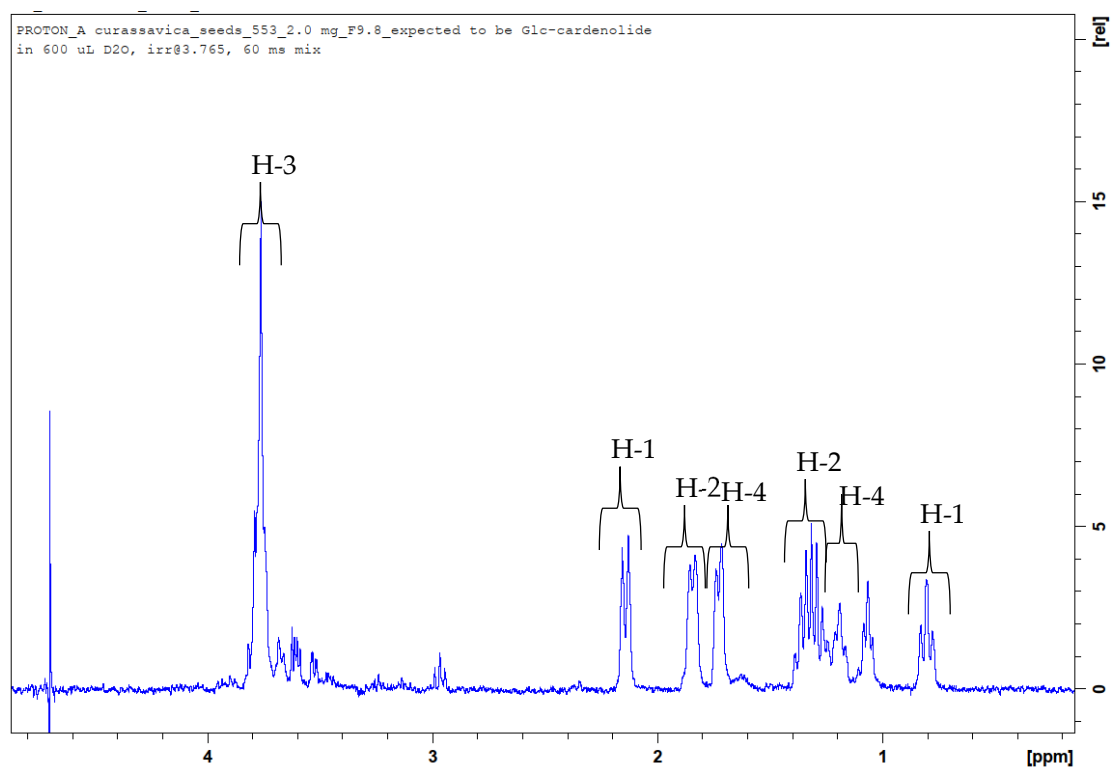


Figure S15: HR-ESI-MS spectrum of compound 2.

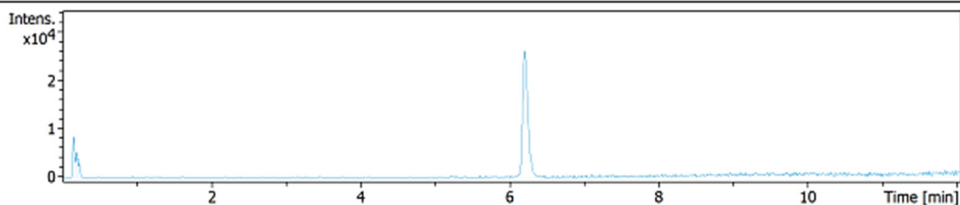
Compound Spectrum List Report

Analysis Info

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 Sample Name alloglucoroglaui 17.3.22 dilu 2
 Comment
 Operator MPI Jena
 Instrument compact 8255754.20124

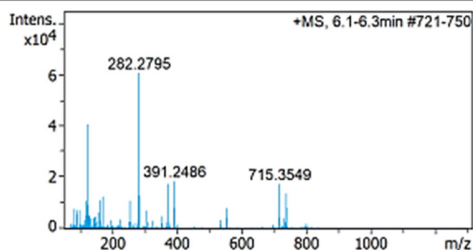
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Scan End	1300 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C



#	RT [min]	Area	Int. Type	I	S/N	Trace	Max. m/z	FWHM [min]
5	6.2	0		60745		EIC 715.3553 +All MS	282.2795	

Cmpd 5, 6.2 min



#	m/z	Res.	S/N	I	I %	FWHM
1	123.0556	9465	576363.1	40302	66.3	0.0130
2	163.1235	9869	159008.9	11119	18.3	0.0165
3	173.0790	9853	178719.4	12497	20.6	0.0176
4	282.2795	10774	868709.9	60745	100.0	0.0262
5	283.2826	10561	177397.7	12405	20.4	0.0268
6	284.2947	10235	183544.4	12834	21.1	0.0278
7	373.2380	10655	249696.2	17460	28.7	0.0350
8	391.2486	10880	264242.6	18477	30.4	0.0360
9	715.3549	11565	250492.6	17516	28.8	0.0619
10	737.3366	11616	196734.8	13757	22.6	0.0635

Figure S16. UV spectrum of compound 2.

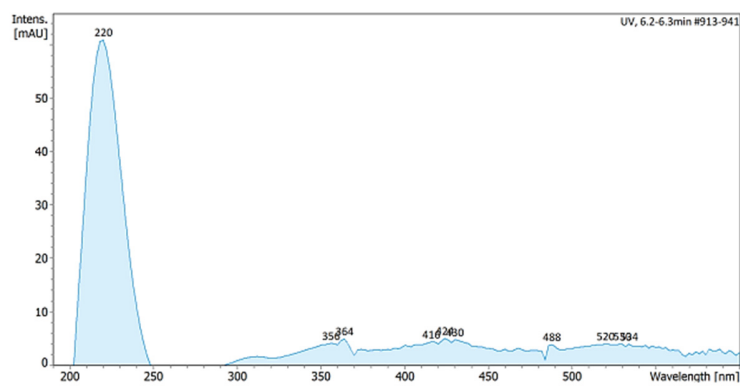
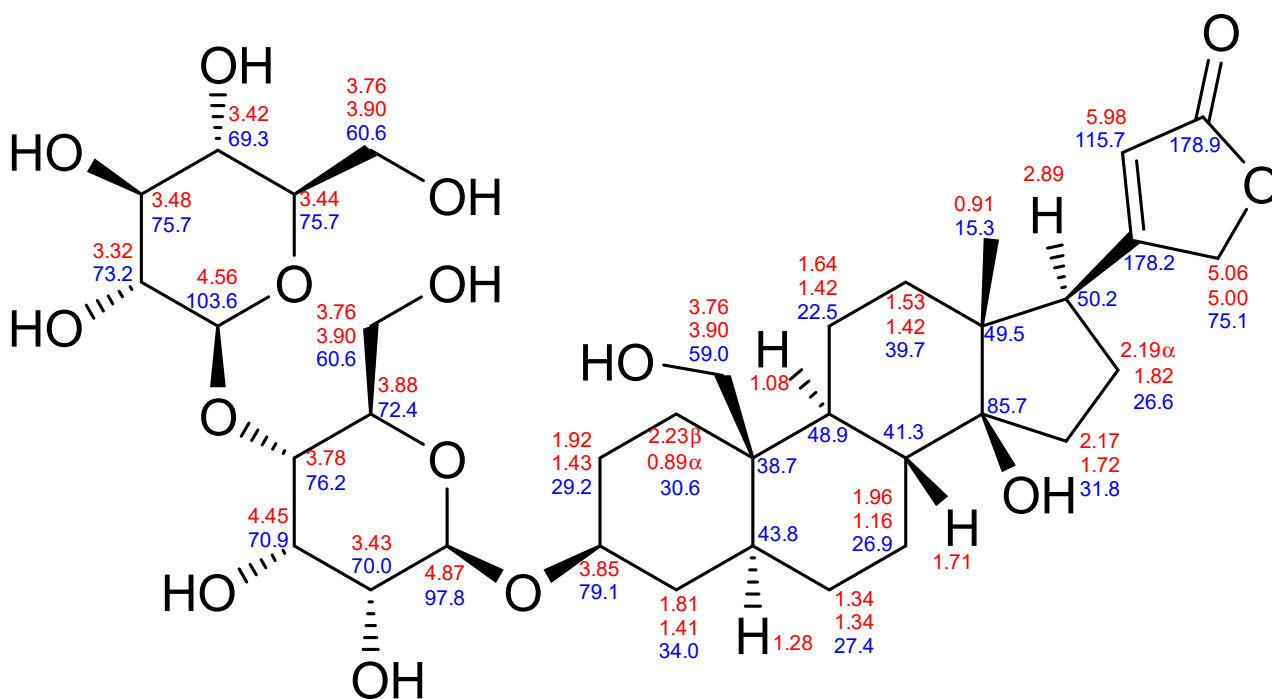


Figure S17: Compound 2-NMR shift assignment



3β-[(4-O-β-D-glucopyranosyl-β-D-allopyranosyl)oxy]-14,19-dihydroxy-5α-card-20(22)-enolide

Figure S18. ¹H-NMR spectrum (500 MHz, D₂O) of compound 2.

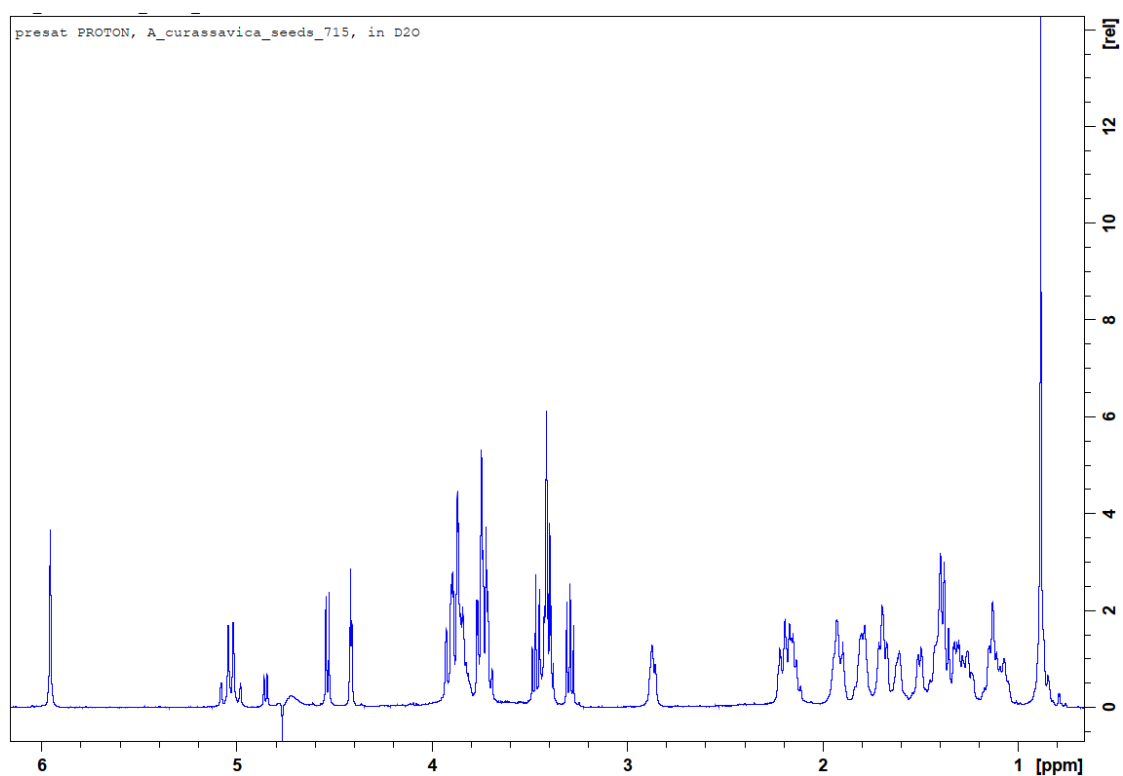


Figure S19. DEPTQ spectrum (125 MHz, D₂O) of compound 2.

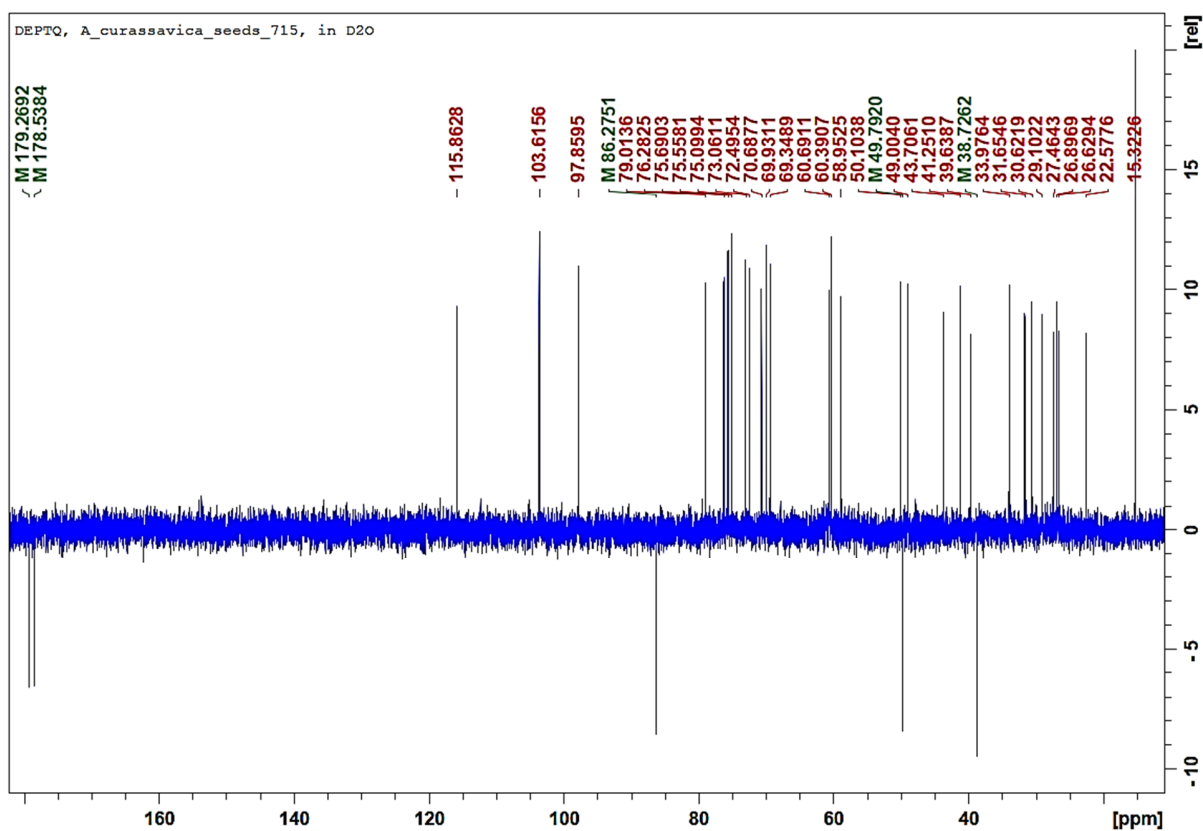


Figure S20. HSQC spectrum (500 MHz, D₂O) of compound 2.

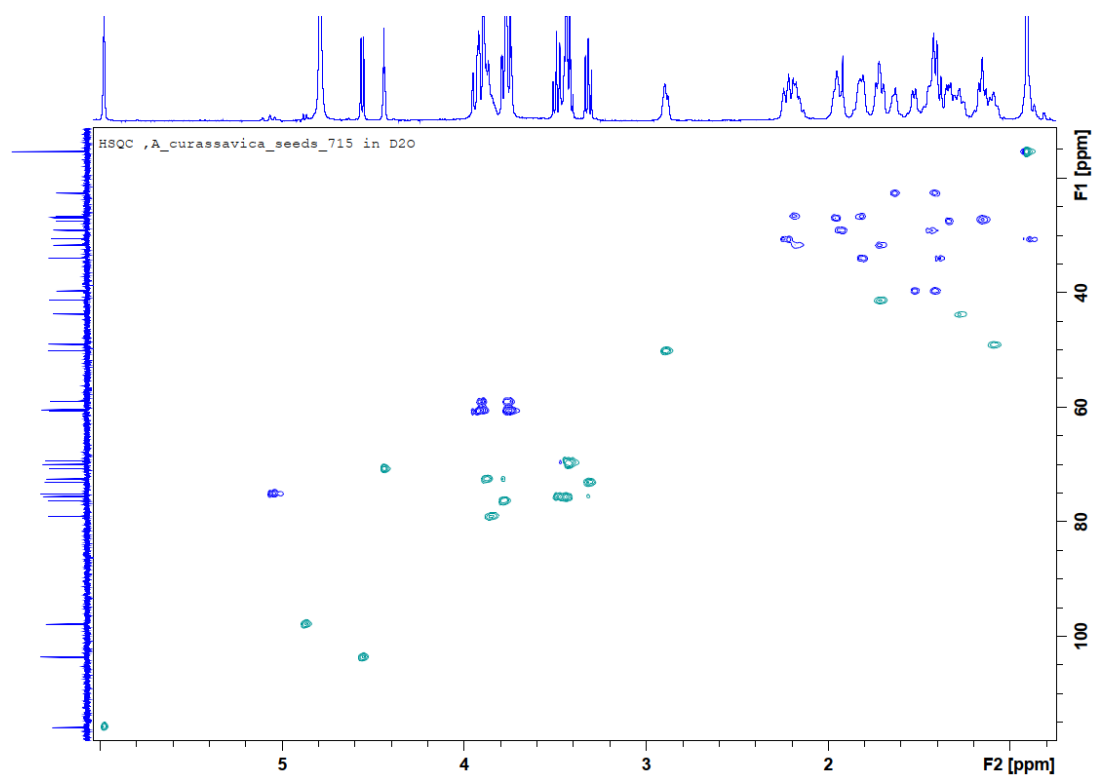


Figure S21. ¹H-¹H COSY spectrum (500 MHz, D₂O) of compound 2.

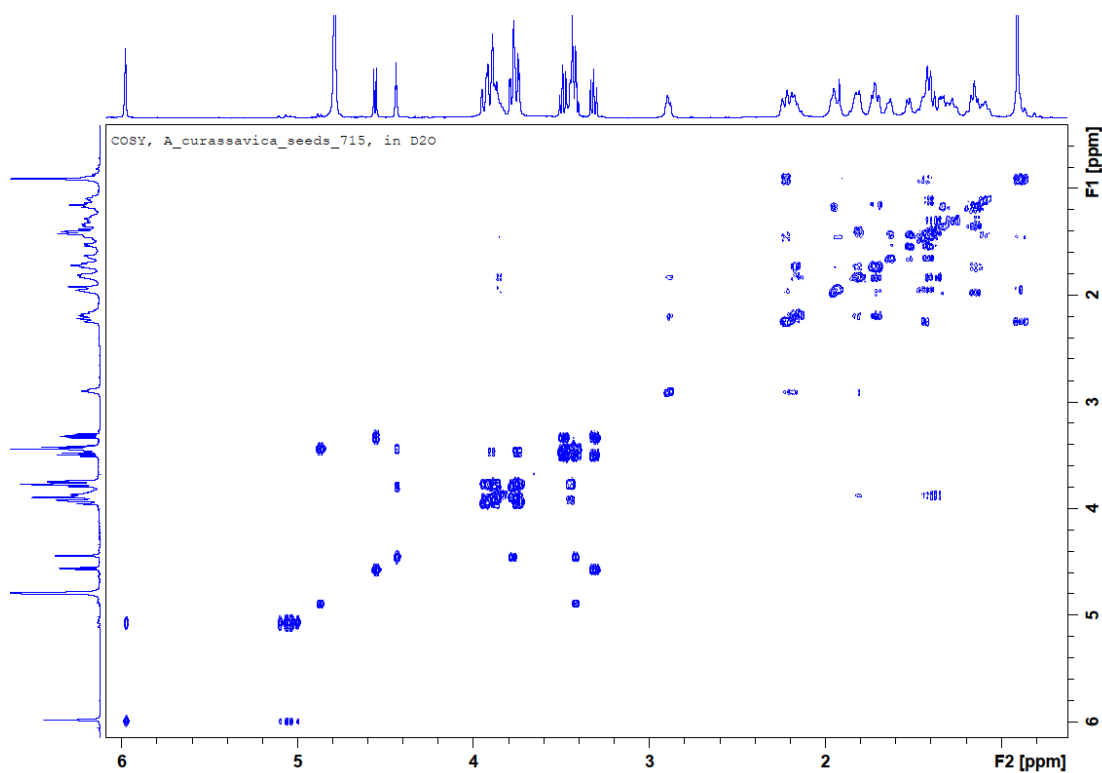


Figure S22. ^1H - ^{13}C HMBC spectrum (500 MHz, D_2O) of compound 2.

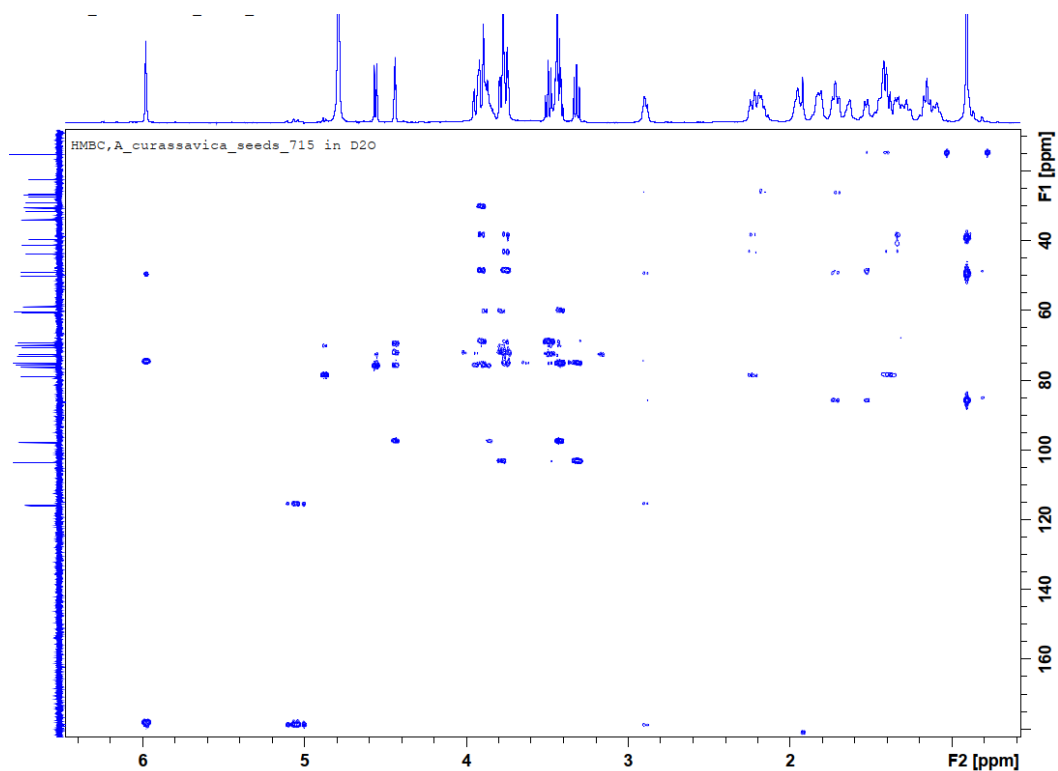


Figure S23. ROESY spectrum (500 MHz, D_2O) of compound 2.

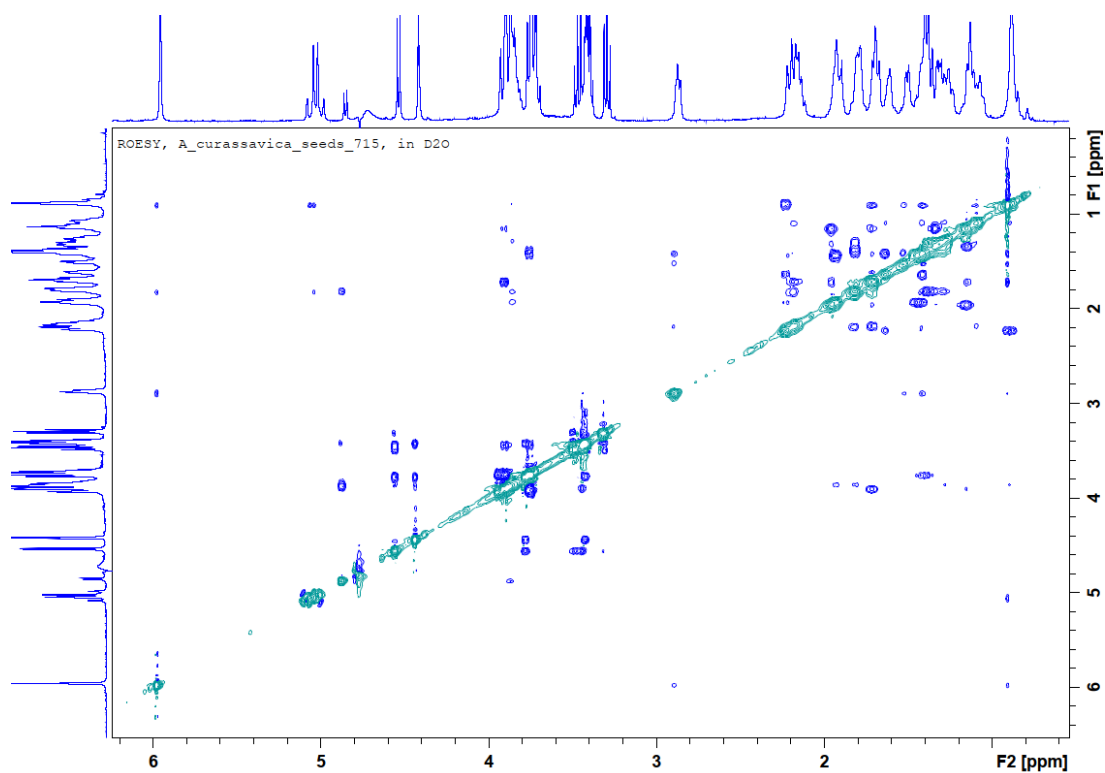


Figure S24. HSQC-TOCSY spectrum (500 MHz, D₂O) of compound **2**.

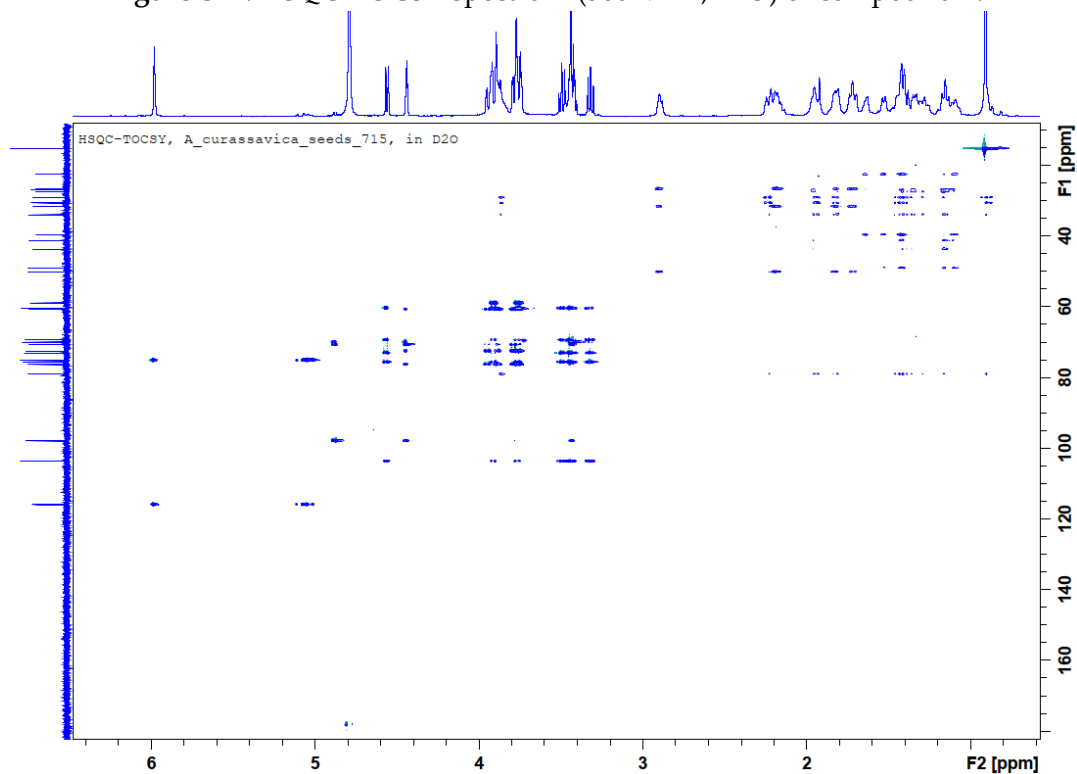


Figure S25. SELTOCSY spectrum (500 MHz, transmitter frequency at 4.87 ppm (H-1'), D₂O) of compound **2**.

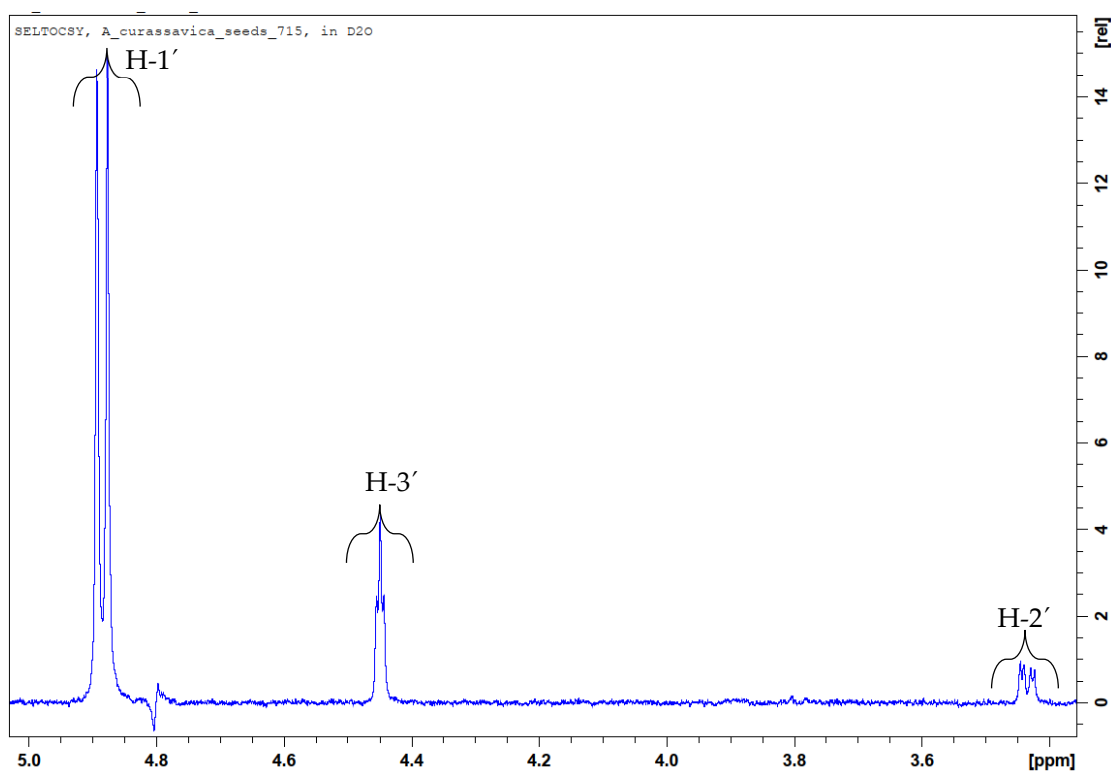


Figure S26. SELTOCSY spectrum (500 MHz, transmitter frequency at 4.45 ppm (H-1''), D₂O) of compound **2**.

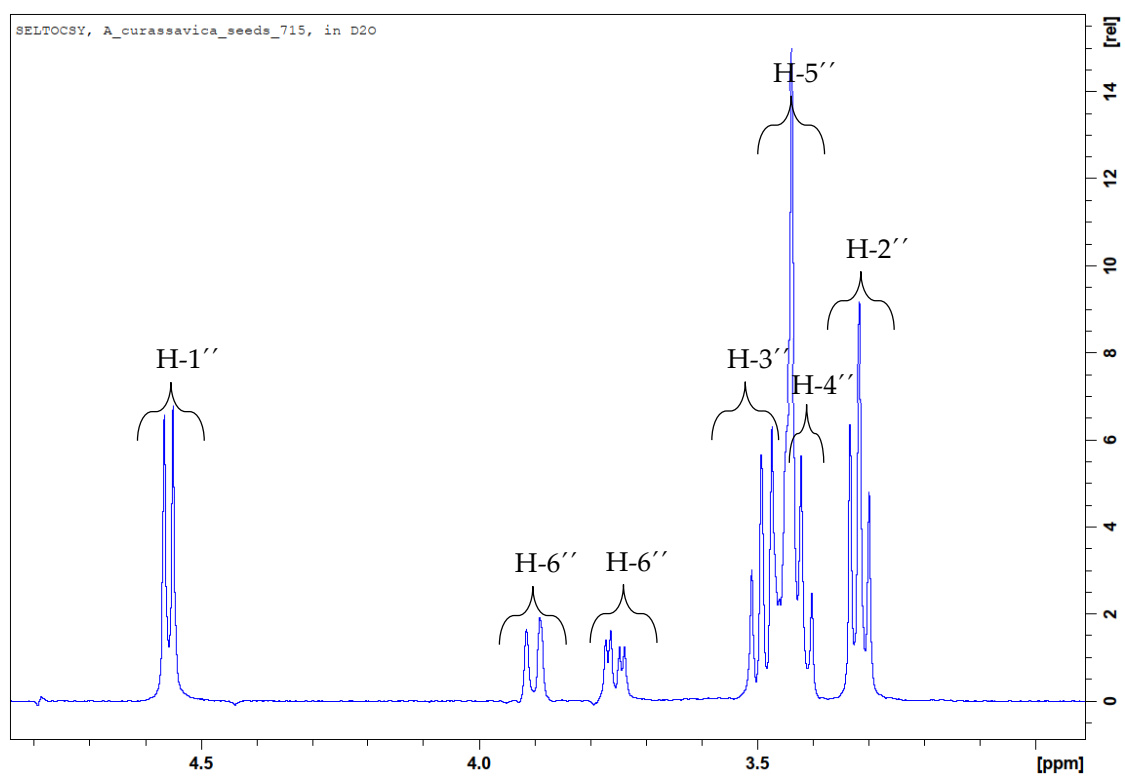


Figure S27: HR-ESI-MS spectrum of compound 3.

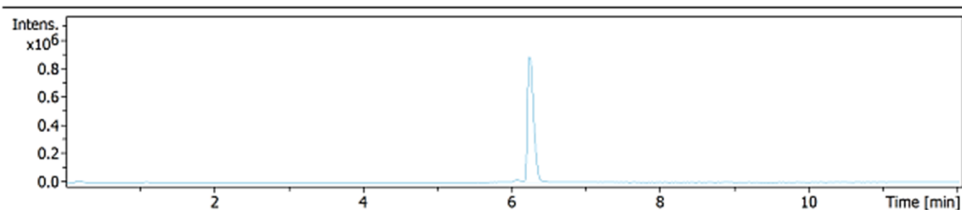
Compound Spectrum List Report

Analysis Info

Acquisition Date 18/03/2022 03:06:11
 Analysis Name \\filer\bird-lab\Users\Paola R-B\LCMS data\Asclepias extracts\693\OHglucocalotrop 17.3.22 dilu
 2_P1-E-5_01_250.d
 Method paola_lcms_pos_checkuplc040221.m Operator MPI Jena
 Sample Name OHglucocalotrop 17.3.22 dilu 2 Instrument compact 8255754.20124
 Comment

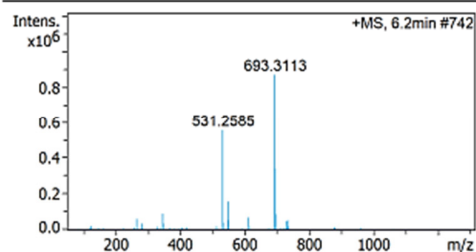
Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.8 Bar
Focus	Not active	Set Capillary	4500 V	Set Dry Heater	220 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	9.0 l/min
Scan End	1300 m/z	Set Charging Voltage	2000 V	Set Divert Valve	Waste
		Set Corona	0 nA	Set APCI Heater	0 °C



#	RT [min]	Area	Int. Type	I	S/N	Trace	Max. m/z	FWHM [min]
1	6.2	0	870479	EIC 693.3114 +All MS	693.3113			

Cmpd 1, 6.2 min



#	m/z	Res.	S/N	I	I %	FWHM
1	286.1326	11730	491.6	60863	7.0	0.0227
2	347.1585	12481	741.5	91945	10.6	0.0278
3	531.2585	11463	4492.6	557083	64.0	0.0464
4	532.2620	11322	1450.9	179915	20.7	0.0470
5	549.2693	11554	1278.0	158466	18.2	0.0475
6	612.2844	12184	550.4	68246	7.8	0.0503
7	693.3113	11752	7020.0	870479	100.0	0.0590
8	694.3145	11731	2766.1	342992	39.4	0.0592
9	695.3174	11857	728.1	90286	10.4	0.0586
10	733.3048	11744	426.0	52824	6.1	0.0624

Figure S28: UV spectrum of compound 3.

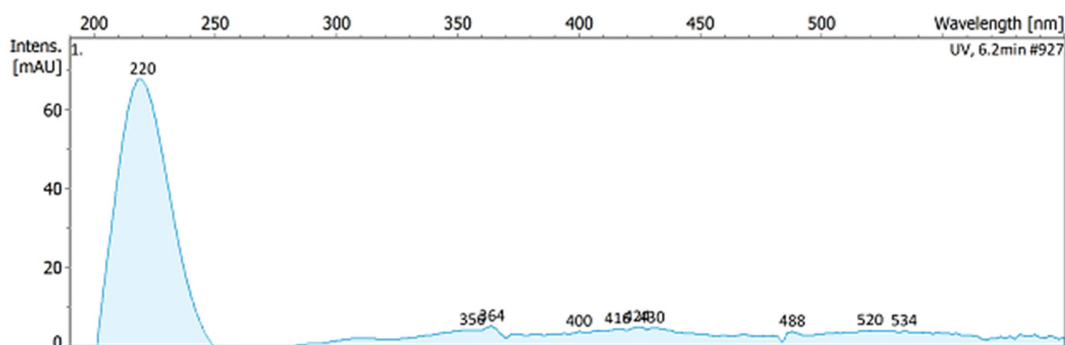
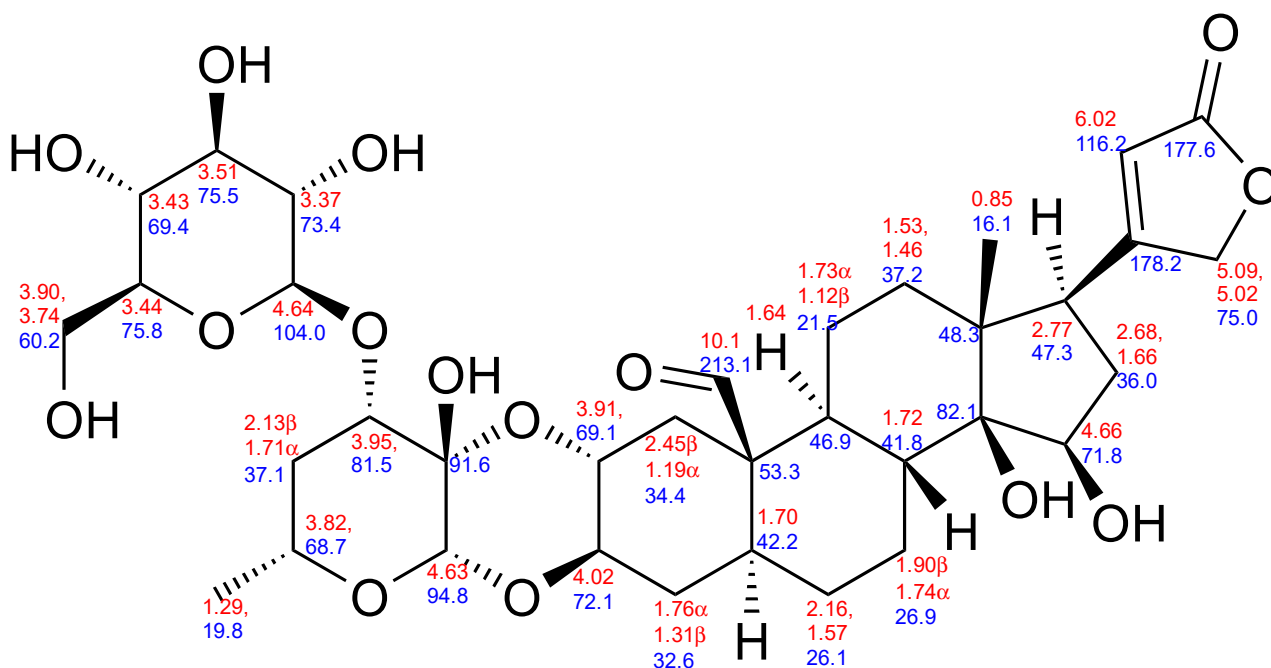


Figure S29: Compound 3-NMR shift assignment



(1*R*,3*R*,3*aS*,3*bR*,6*aR*,7*aS*,9*R*,11*S*,11*aS*,12*aR*,13*aR*,13*bS*,15*aR*)-13*a*-formyl-3,3*a*,11*a*-trihydroxy-9,15*a*-dimethyl-1-(5-oxo-2,5-dihydrofuran-3-yl)icosahydro-1*H*,7*aH*-cyclopenta[7,8]phenanthro[2,3*b*]pyrano[3,2-*e*][1,4]dioxin-11-yl β-*L*-glucopyranoside

Figure S30. ¹H-NMR spectrum (500 MHz, D₂O) of compound 3.

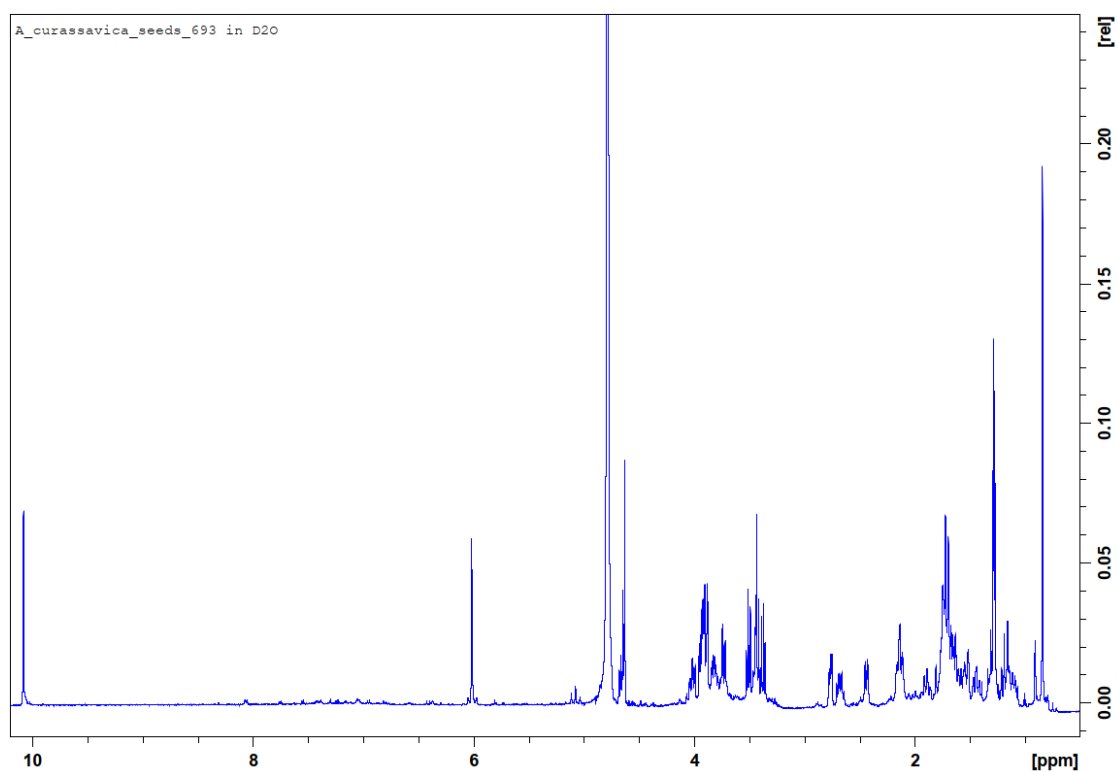


Figure S31. DEPTQ spectrum (125 MHz, D₂O) of compound 3.

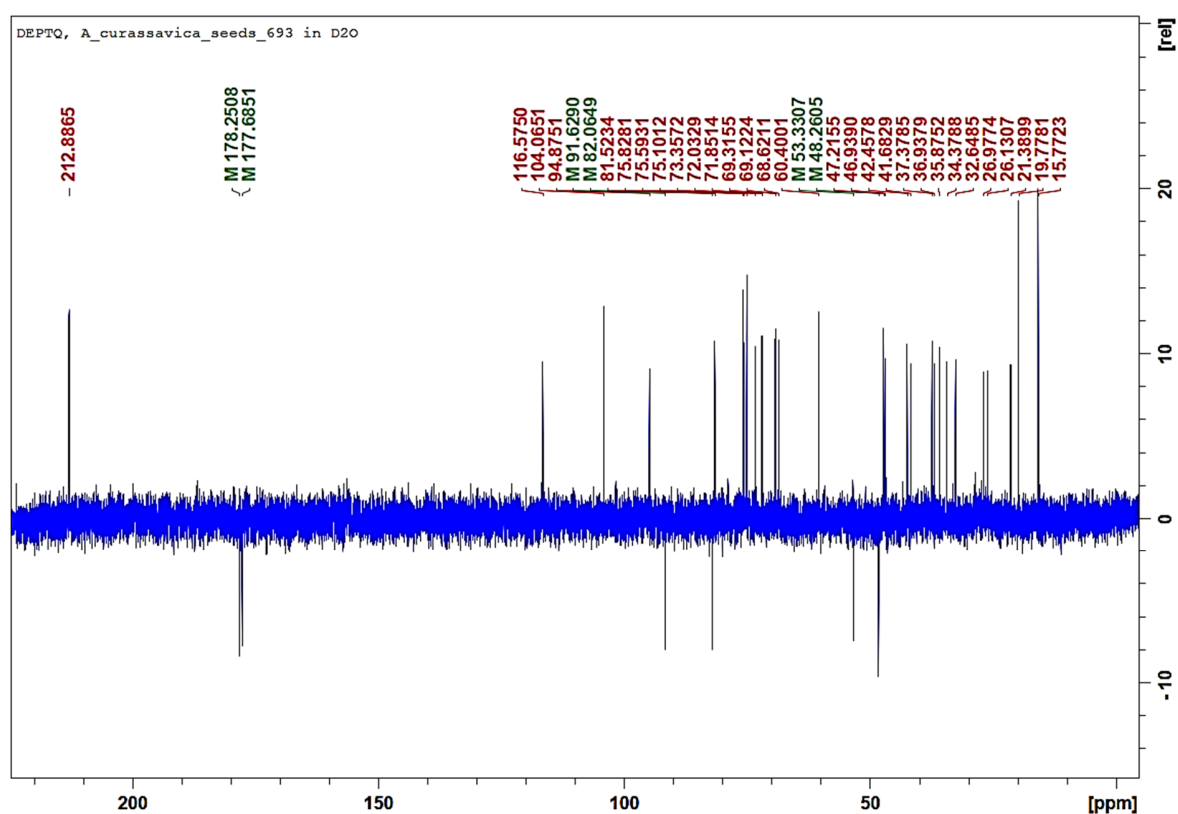


Figure S32. HSQC spectrum (500 MHz, D₂O) of compound 3.

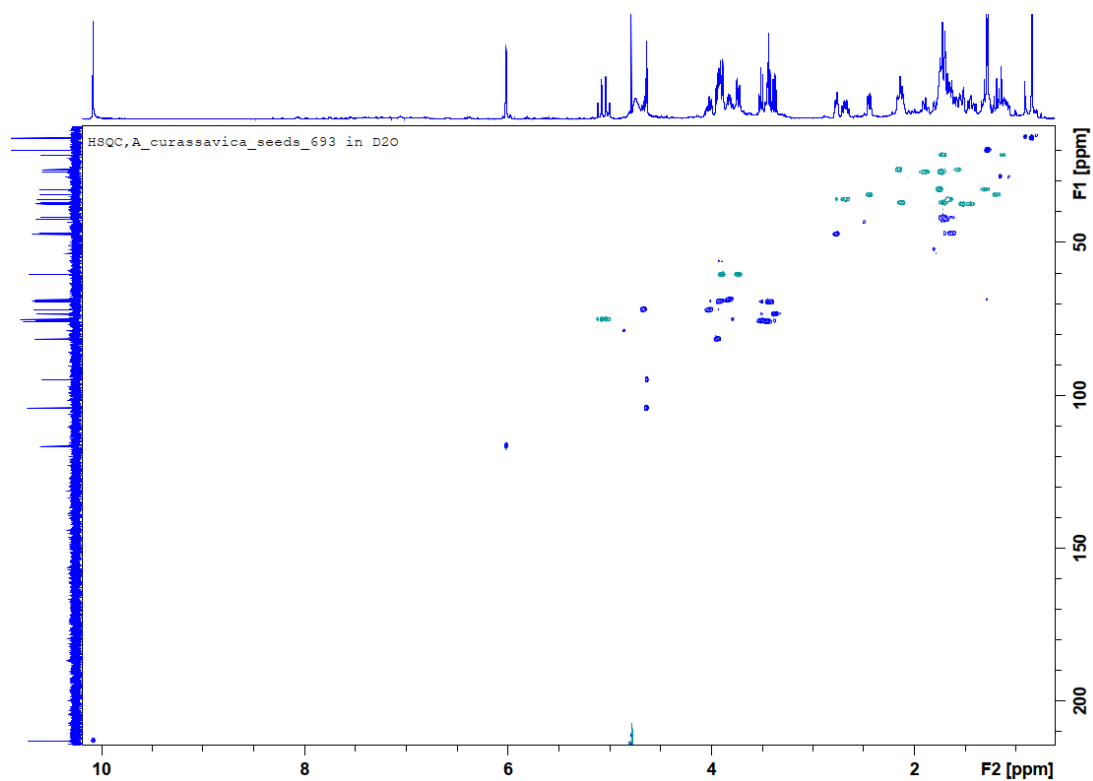


Figure S33. ^1H - ^1H COSY spectrum (500 MHz, D_2O) of compound 3.

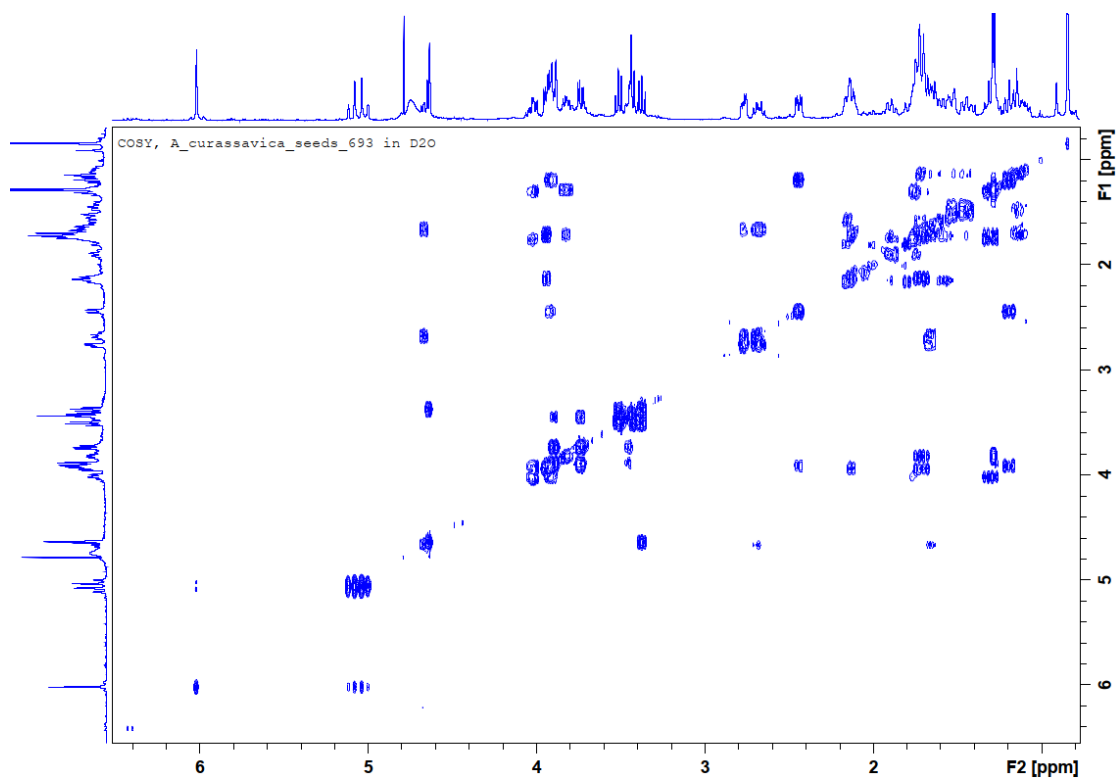


Figure S34. ^1H - ^{13}C HMBC spectrum (500 MHz, D_2O) of compound 3.

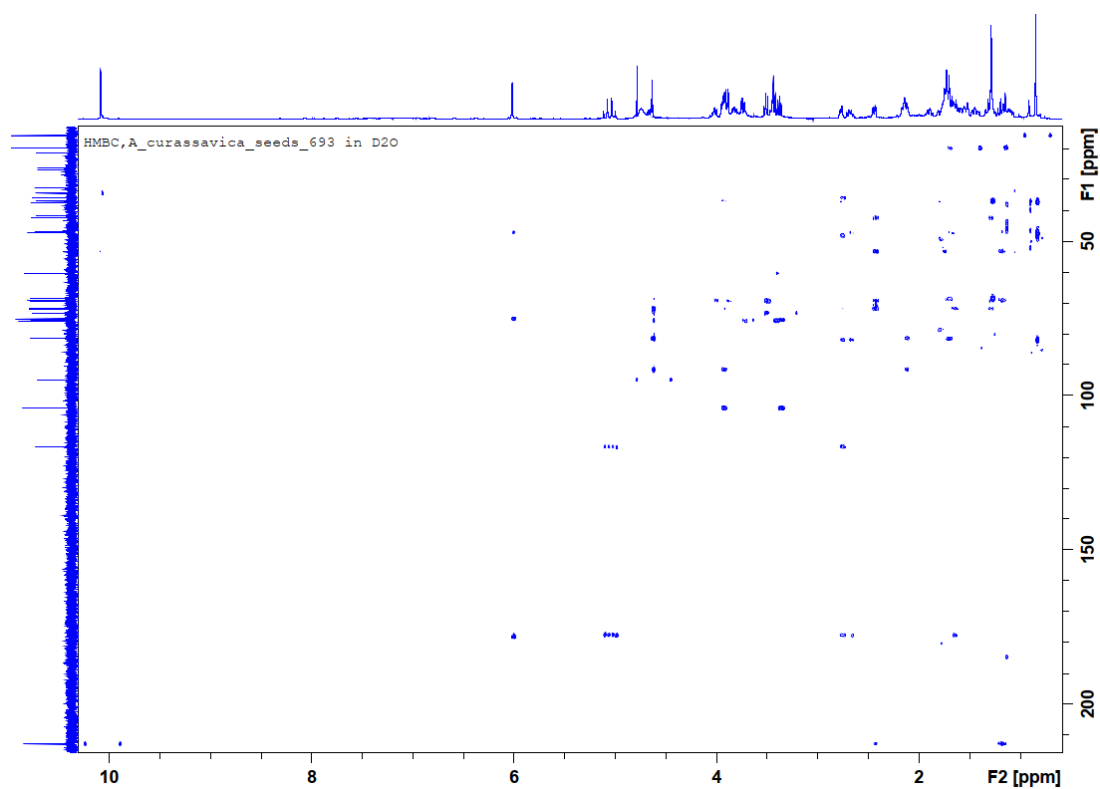


Figure S35. ROESY spectrum (500 MHz, D₂O) of compound 3.

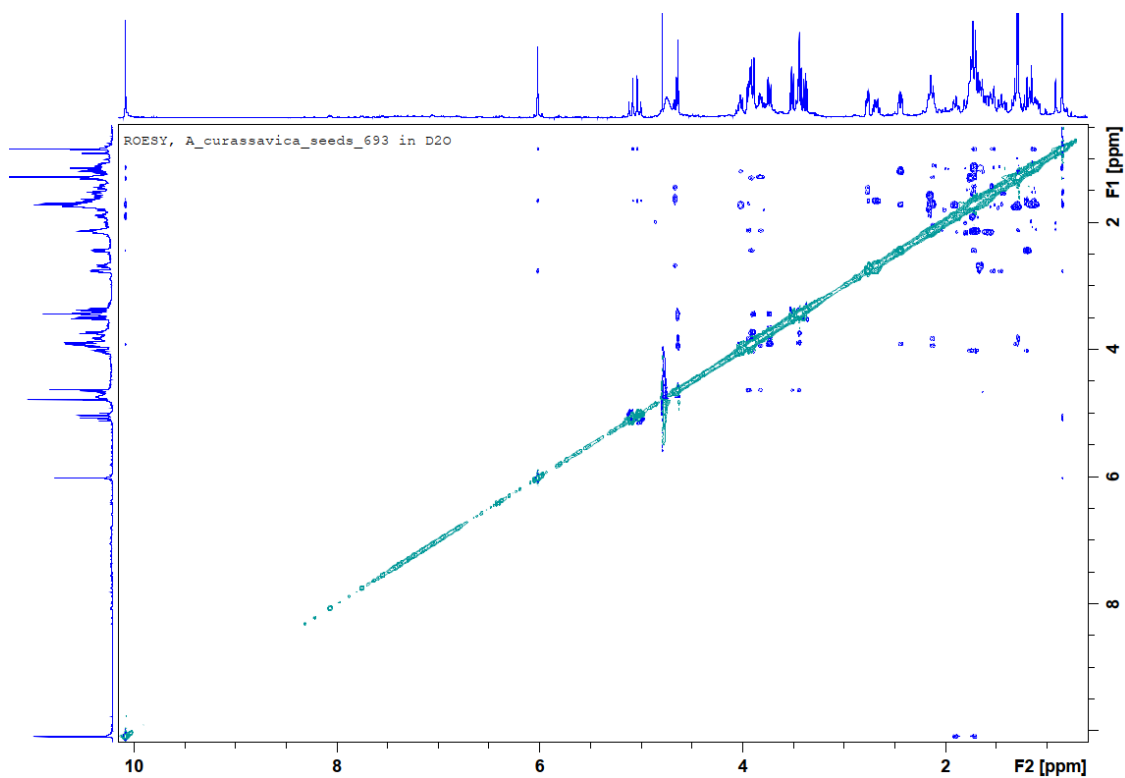


Figure S36. HSQC-TOCSY spectrum (500 MHz, D₂O) of compound 3.

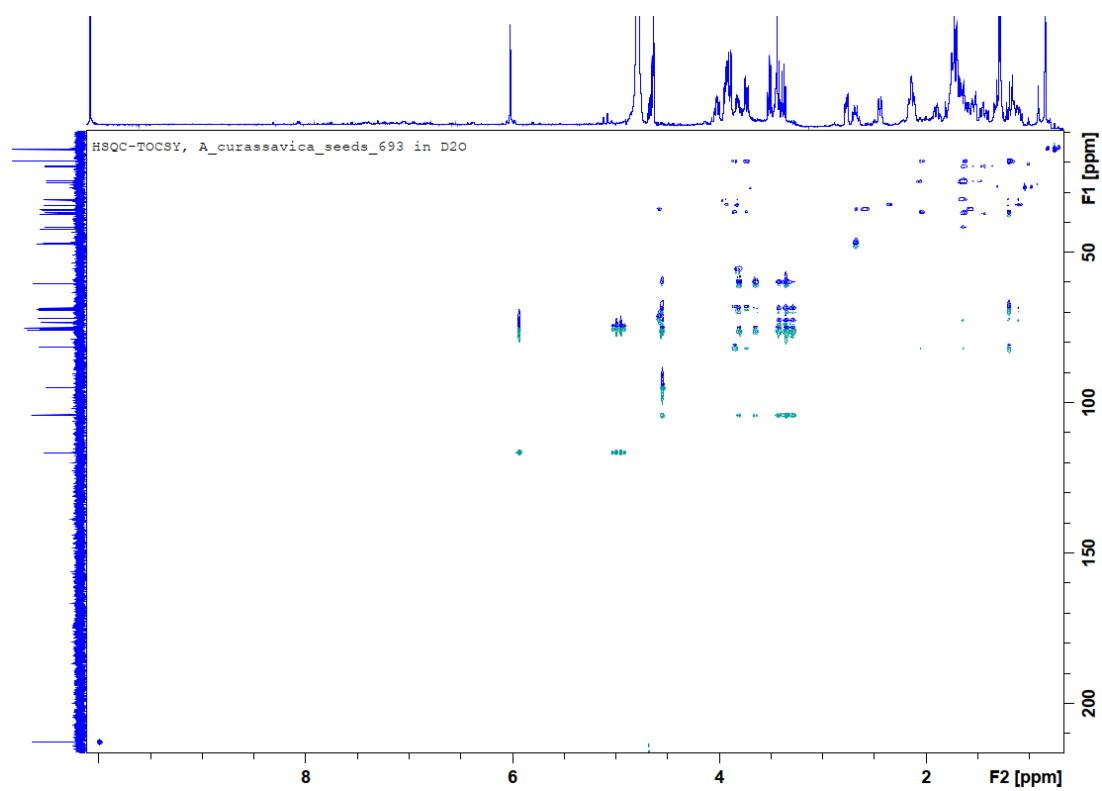


Figure S37: HR-ESI-MS spectrum of compound 4.

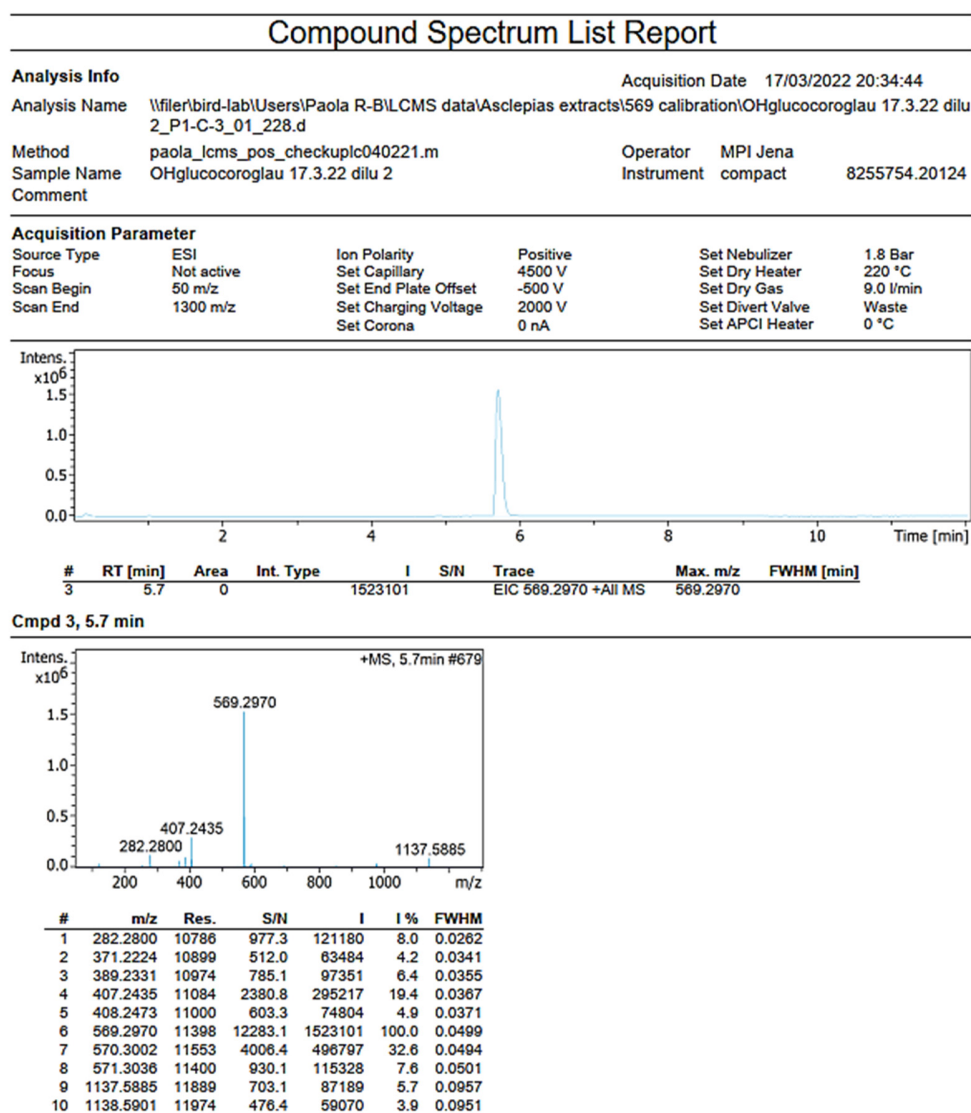


Figure S38: UV spectrum of compound 4.

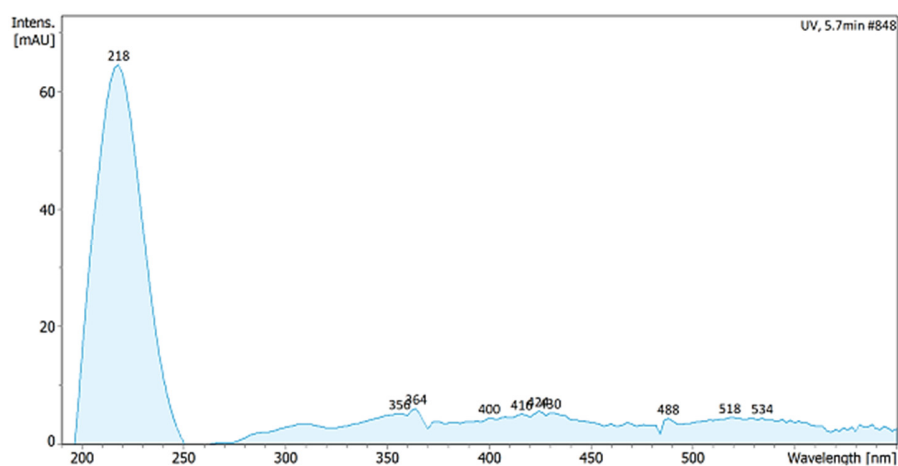
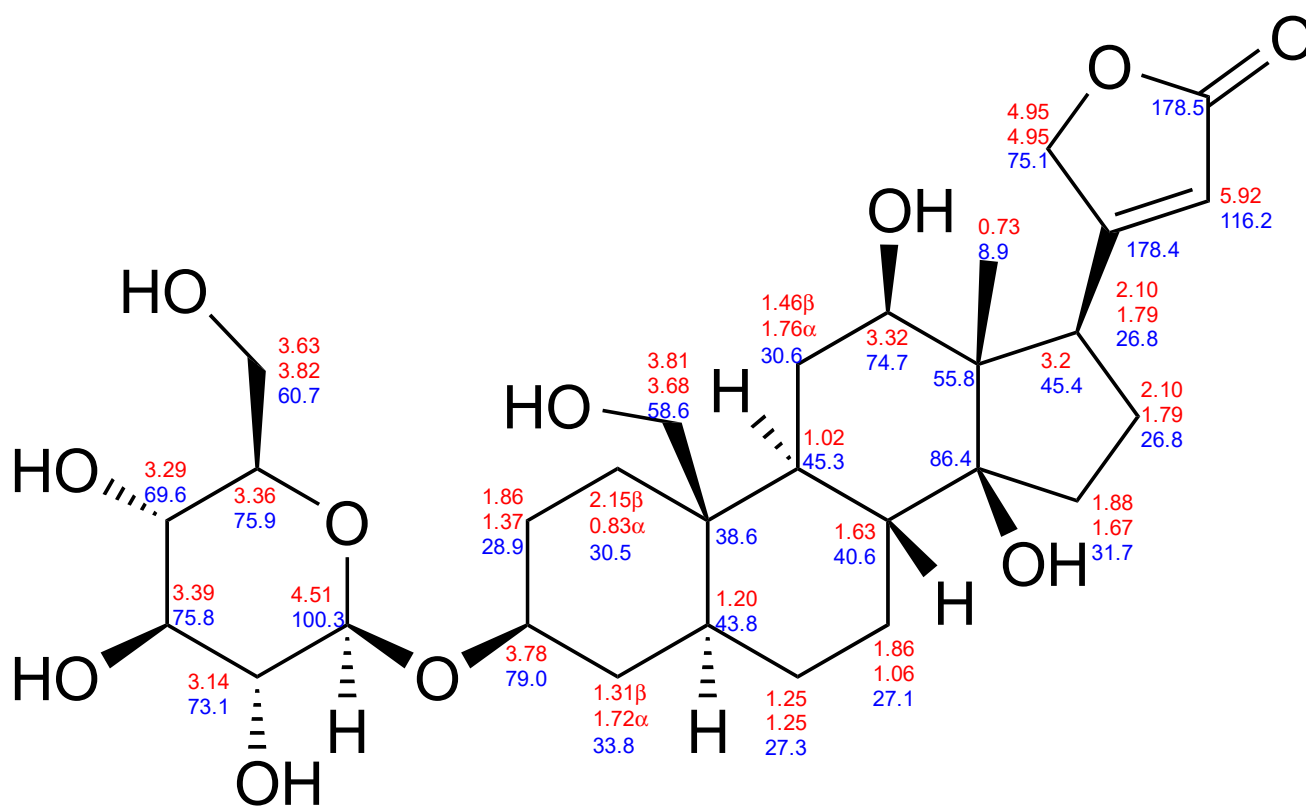


Figure S39: Compound 4-NMR shift assignment



3β-(β-D-glucopyranosyloxy)-12β,14,19-trihydroxy-5α-card-20(22)-enolide

Figure S40. ¹H-NMR spectrum (500 MHz, D₂O) of compound 4.

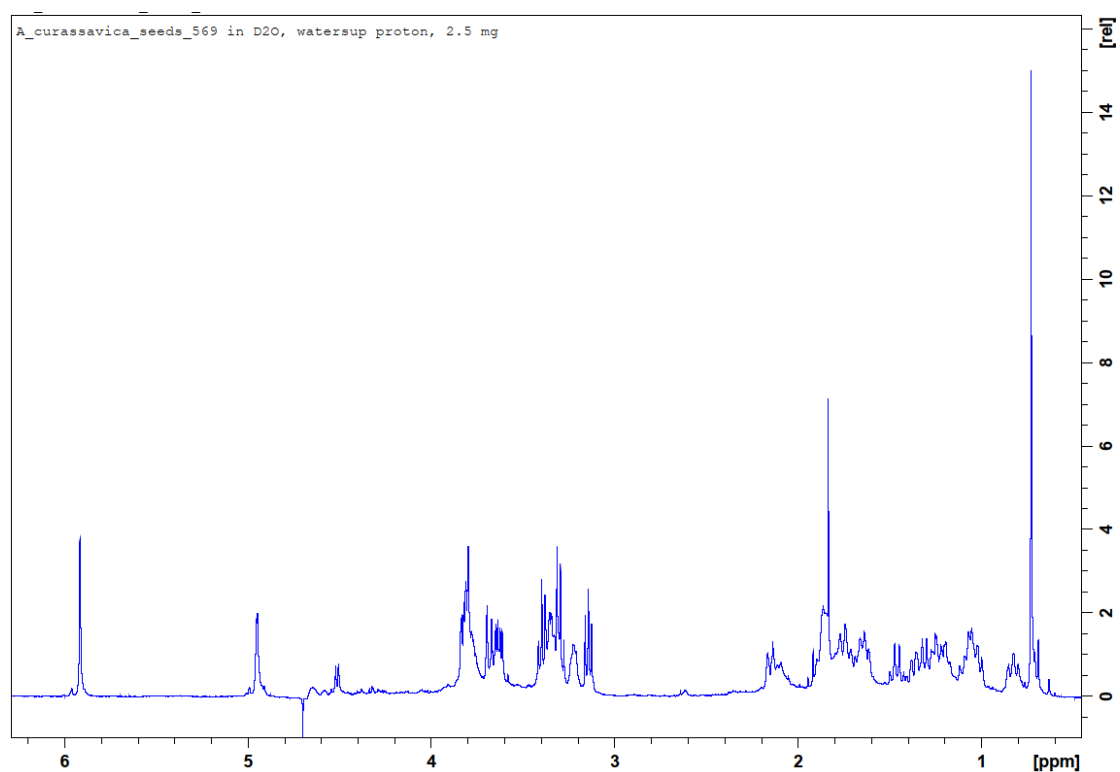


Figure S41. APT spectrum (125 MHz, D₂O) of compound 4.

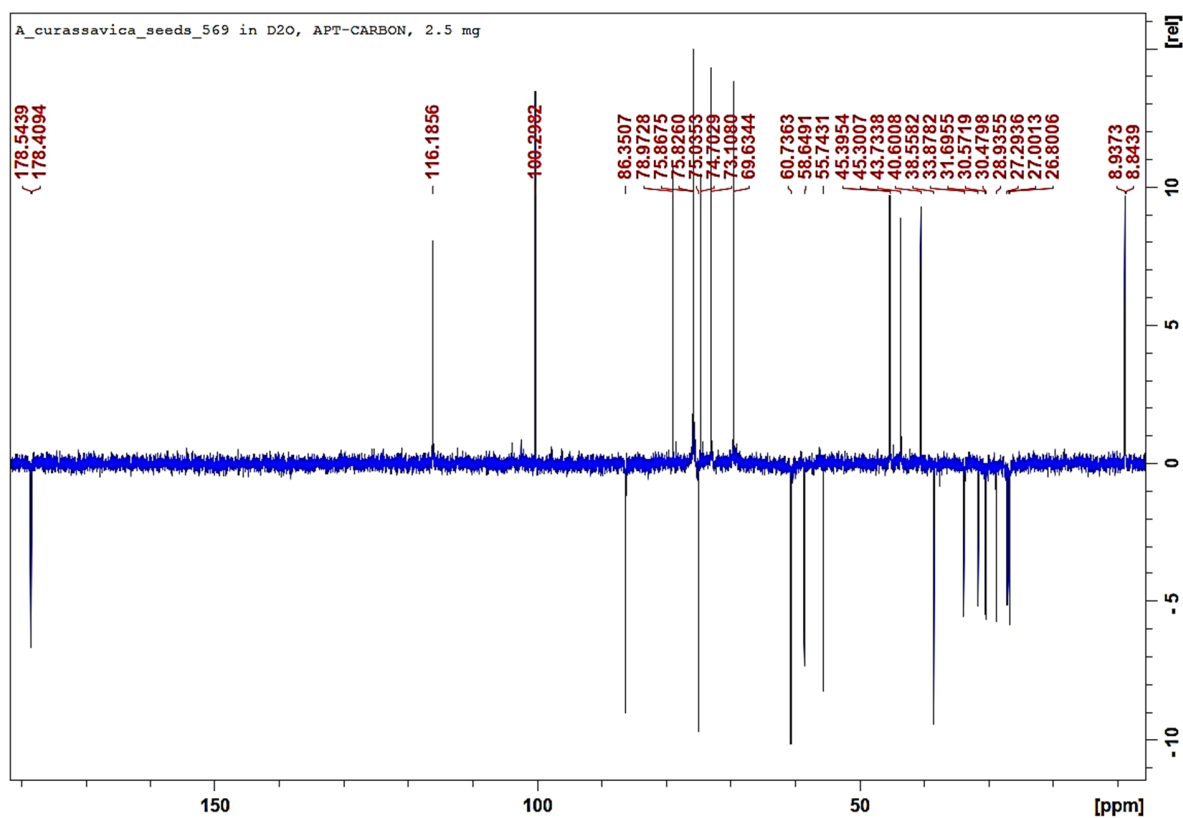


Figure S42. HSQC spectrum (500 MHz, D₂O) of compound 4.

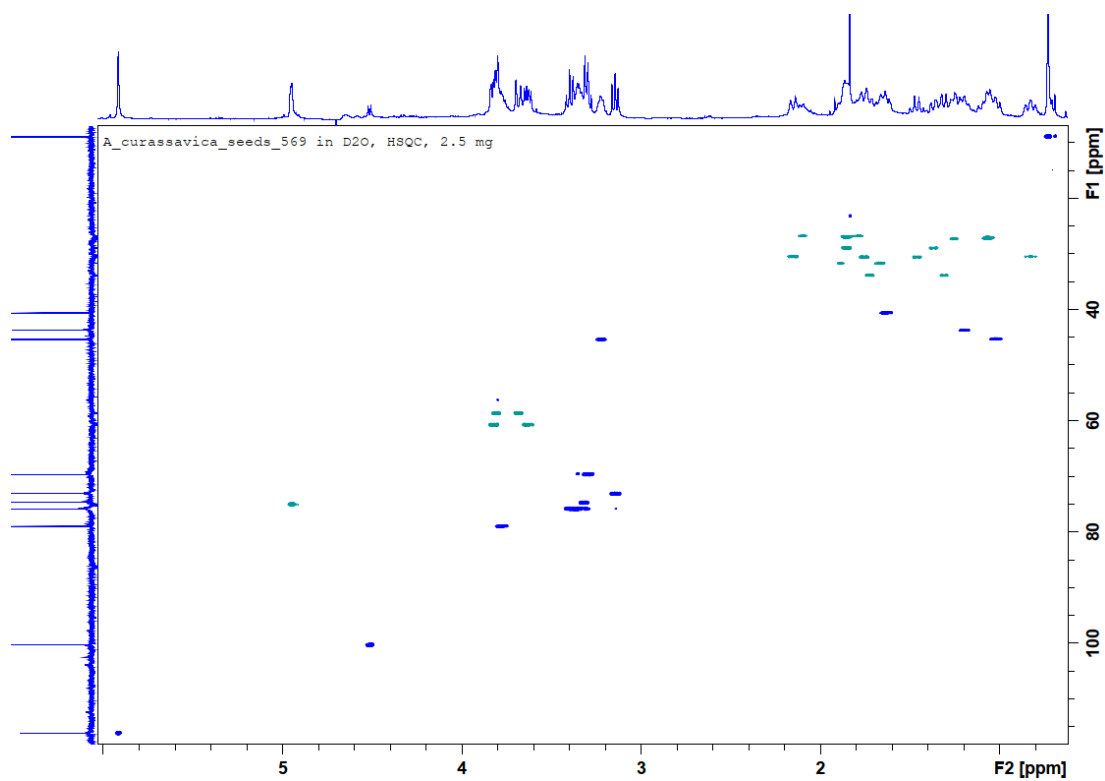


Figure S43. ^1H - ^1H COSY spectrum (500 MHz, D_2O) of compound 4.

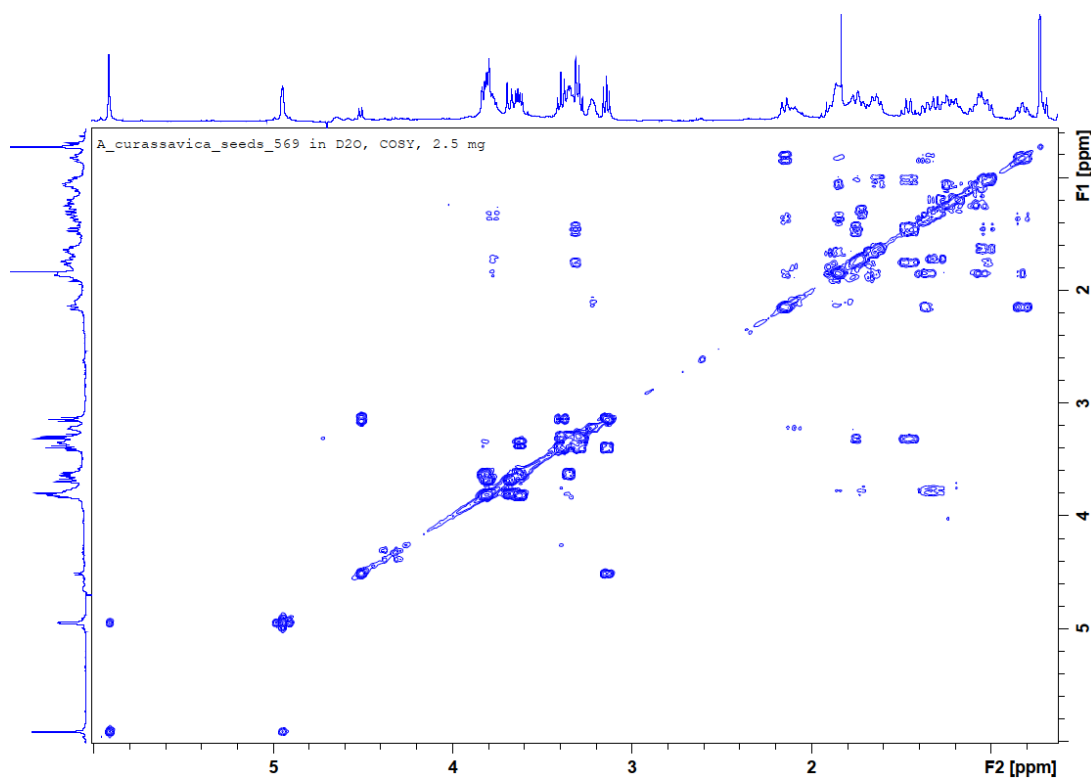


Figure S44. ^1H - ^{13}C HMBC spectrum (500 MHz, D_2O) of compound 4.

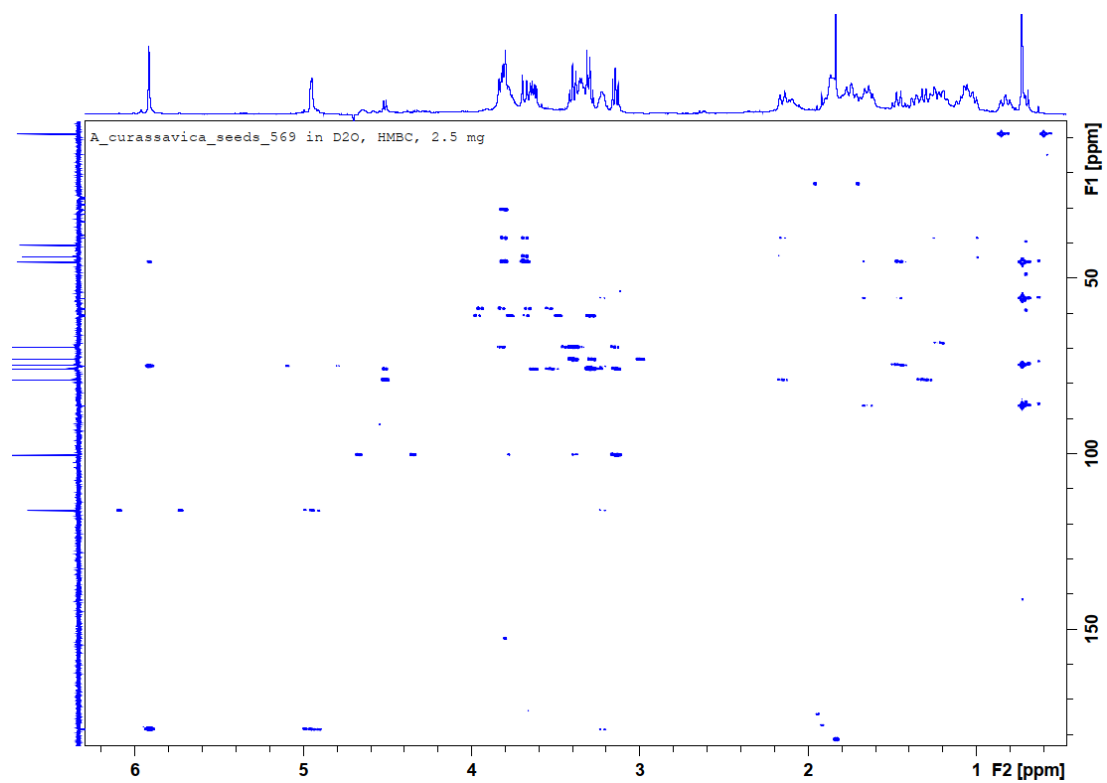


Figure S45. ROESY spectrum (500 MHz, D₂O) of compound 4.

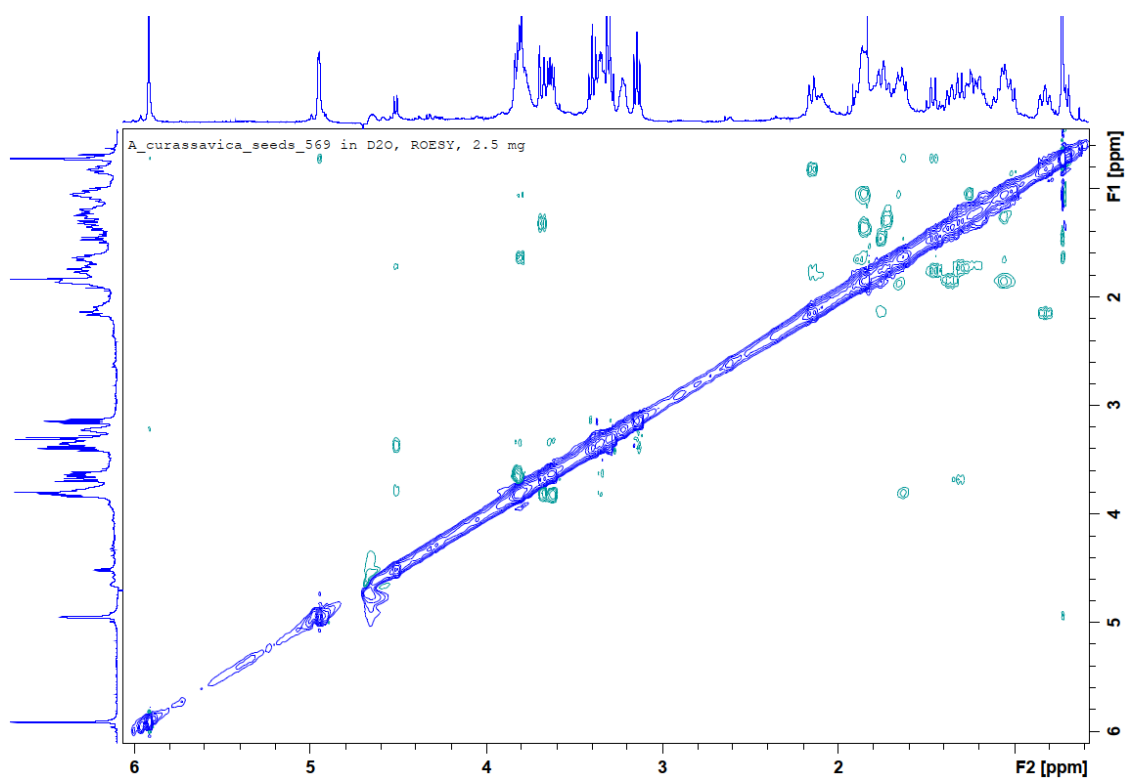


Figure S46. HSQC-TOCSY spectrum (500 MHz, D₂O) of compound 4.

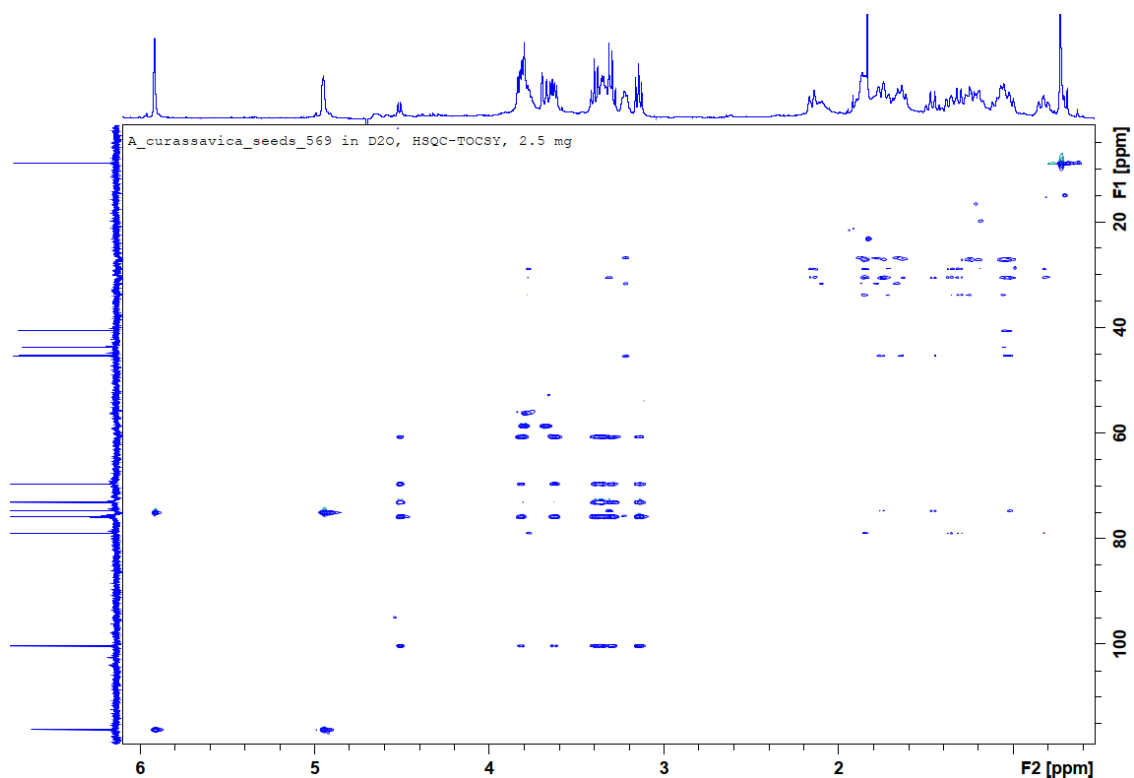


Figure S47. SELTOCSY spectrum (500 MHz, transmitter frequency at 4.514 ppm, D₂O) of compound 4.

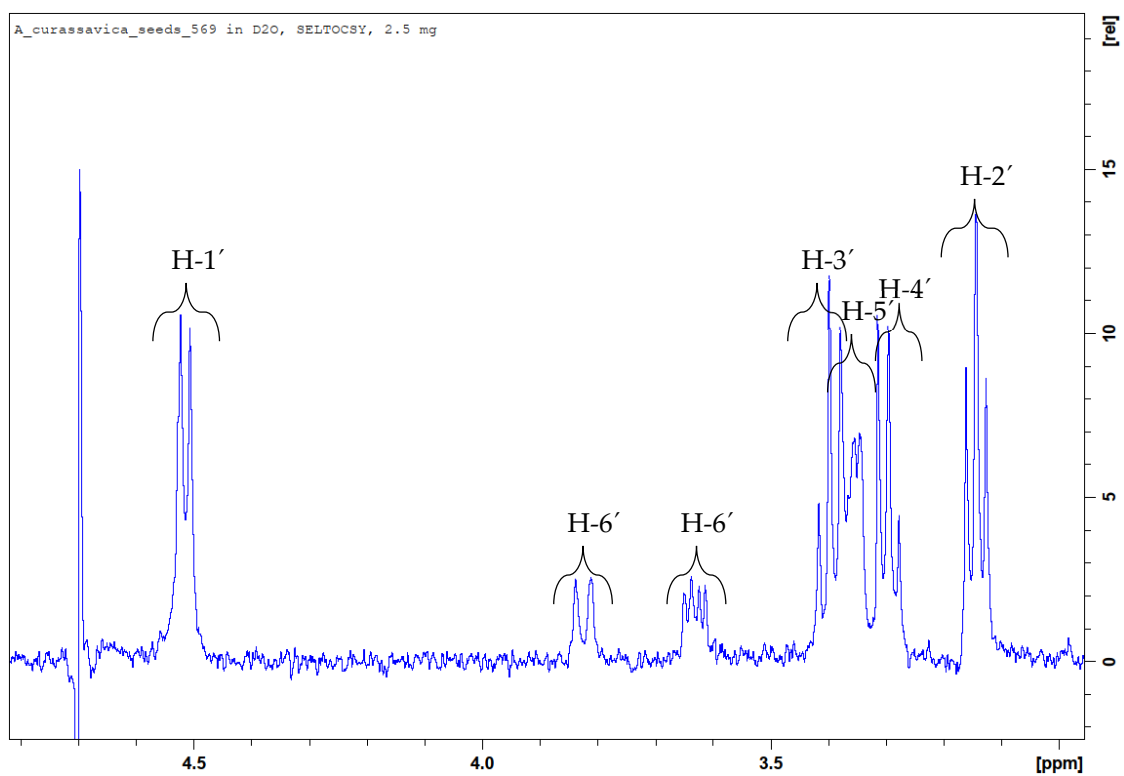


Figure S48. SELNOESY spectrum (500 MHz, transmitter frequency at 3.764 ppm (H-3), D₂O) of compound 4.

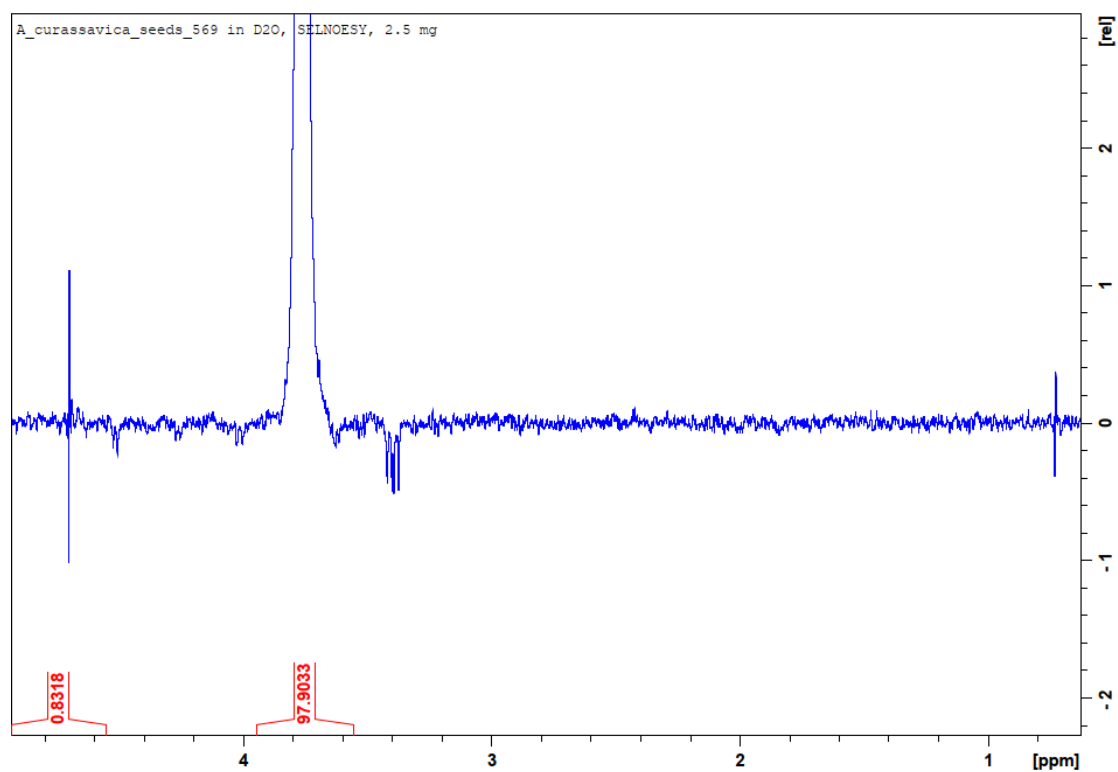


Figure S49. SELNOESY spectrum (500 MHz, transmitter frequency at 3.223 ppm (H-17), D₂O) of compound **4**.

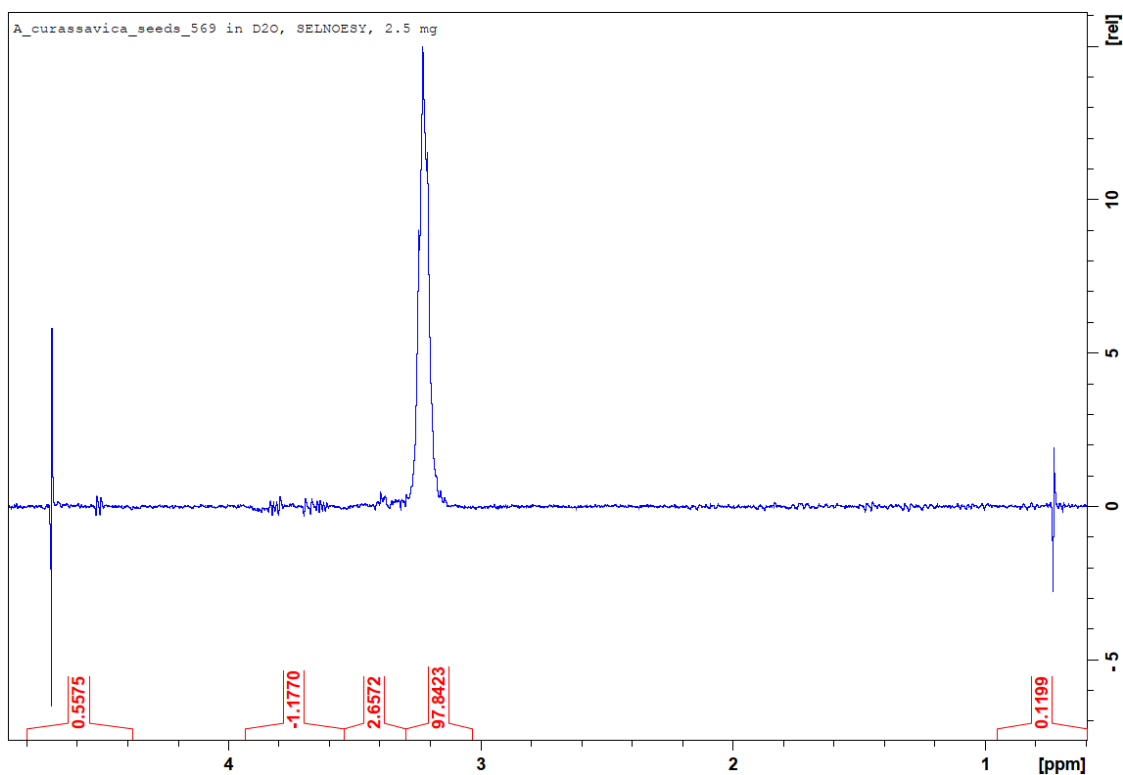


Table S2: Cardenolides quantification (mg/g of seeds)

Compound	Concentration (mg /g of seeds)
1	0.014 (+/- 0.0062)
2	0.063 (+/- 0.0038)
3	0.053 (+/- 0.0029)
4	0.004 (+/- 0.0009)
5	4.503 (+/- 0.2630)
6	2.065 (+/- 0.1930)
7	0.733 (+/- 0.0724)
8	0.810 (+/- 0.1570)
9	0.275 (+/- 0.1040)
10	0.457 (+/- 0.3020)

Figure S50: Inhibition curves of *Sus domesticus* Na⁺/K⁺ ATPase by compounds 1,2, 5–10 and ouabain

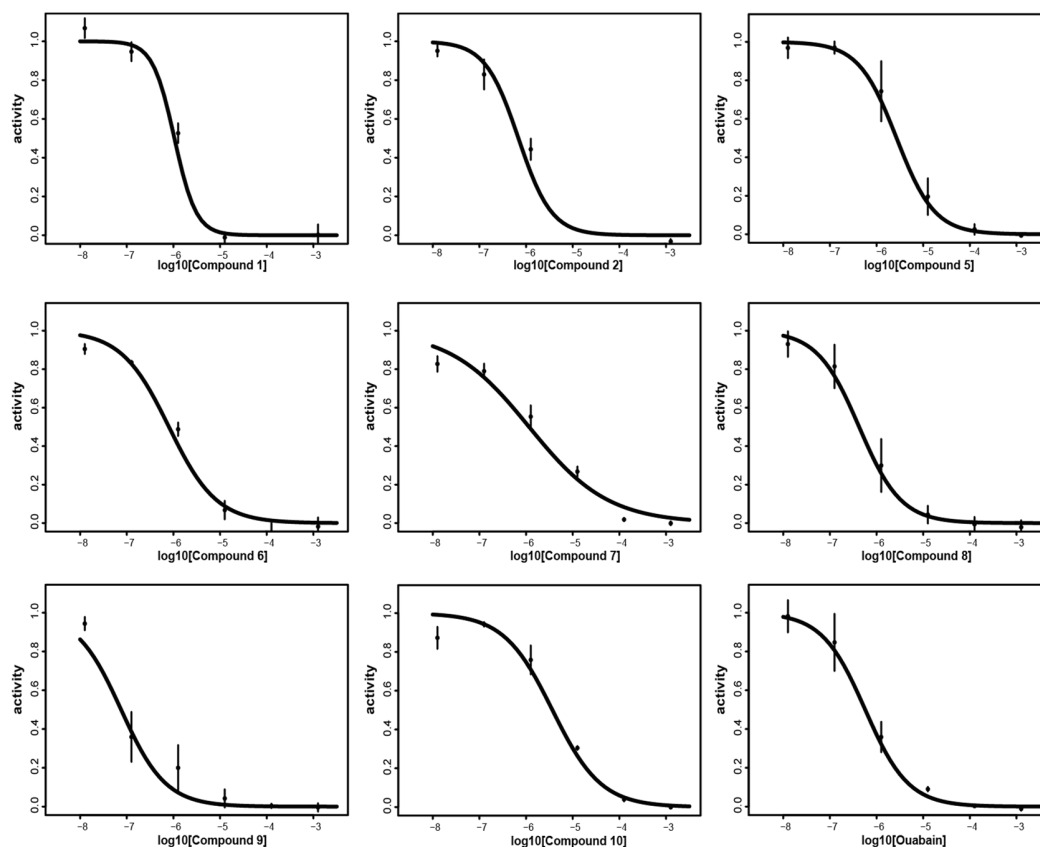


Table S3: IC₅₀ values (μM) of compounds 1,2,5–10 and ouabain against porcine Na⁺/K⁺ ATPase.

Compound	IC ₅₀ [μM]
1	1.01 x10 ⁻⁶ (+/- 1.20 x10 ⁻⁸)
2	7.42 x10 ⁻⁷ (+/- 2.60 x10 ⁻⁸)
5	2.98 x10 ⁻⁶ (+/- 1.69 x10 ⁻⁷)
6	8.09 x10 ⁻⁷ (+/- 8.00 x10 ⁻⁹)
7	1.20 x10 ⁻⁶ (+/- 3.6 x10 ⁻⁸)
8	4.72 x10 ⁻⁷ (+/- 3.00 x10 ⁻⁸)
9	9.65 x10 ⁻⁸ (+/- 6.00 x10 ⁻⁹)
10	3.67 x10 ⁻⁶ (+/- 7.00 x10 ⁻⁸)
Ouabain	4.48 x10 ⁻⁷ (+/- 1.1 x10 ⁻⁸)

Table S4: Comparison of IC₅₀ values between compounds **1,2,5–10** along with ouabain, p-values adjusted with Bonferroni method (* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$).

	1	2	5	6	7	8	9	10	Ouabain
1		1.000 ns	0.347 ns	1.000 ns	1.000 ns	0.651 ns	<0.001 ***	0.046 *	1.000 ns
2			0.035 *	1.000 ns	1.000 ns	1.000 ns	<0.001 ***	0.005 **	1.000 ns
5				0.083 ns	0.858 ns	0.001 **	<0.001 ***	1.000 ns	0.002 **
6					1.000 ns	1.000 ns	<0.001 ***	0.011 *	1.000 ns
7						0.260 ns	<0.001 ***	0.119 ns	0.537 ns
8							0.005 **	<0.001 ***	1.000 ns
9								<0.001 ***	0.002 **
10									<0.001 ***