SUPPORTING INFORMATION

Noncyanogenic Cyanoglucoside Cyclooxygenase Inhibitors from *Simmondsia chinensis*

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Abstract

Two new noncyanogenic cyanoglucoside dimers, simmonosides A and B (**1** and **2**), were identified from the aqueous extract of jojoba (*Simmondsia chinensis*) leaves. **1** and **2** are the first examples of noncyanogenic cyanoglucoside dimers containing a unique four-membered ring, representing novel dimerization patterns at α,β-unsaturated carbons of a nitrile group in **1** and γ,δ-unsaturated carbons in **2**. Their structures were elucidated based on spectroscopic evidence and electronic circular dichroism (ECD) calculations. Compounds **1** and **2** exhibit promising COX-2 inhibition activity, with IC_{50} values of 13.5 μM and 11.4 μM, respectively.

Content

EXPERIMENTAL SECTION

General Experimental Procedures. IR spectra were recorded on a Nicolet 5700 FT-IR Microscope spectrometer (FT-IR Microscope Transmission). Optical rotations were measured on a Perkin-Elmer Model 343 polarimeter. UV absorption was performed with a UV-vis spectrometer (Cary 50 spectrophotometer). NMR spectra were obtained on a Bruker Avance DRX600 spectrometer. CD spectra were recorded at room temperature on a Jasco J-815 spectrophotometer in 1 cm cuvettes. HPLC separations were carried out using an Agilent Chromatorex Zorbax SB (C18, 9.4 × 250 mm, 5 μm), an Agilent Chromatorex Eclipse XDB (C3, 9.4 × 250 mm, 5 μm) and an Agilent 1200 series gradient pump monitored using a DAD G1315B variable-wavelength UV detector. All flash chromatography was performed on Sepacore Flash Chromatography System, Buchi Labortechnik AG, Netherlands. Column chromatography (CC) was performed using a silica gel (Kieselgel 60Å, 40–63 μM mesh size, Fluorochem, UK), Size exclusion chromatography (Sephadex LH-20, 25–100 mm mesh size, GE Healthcare ioSciences AB). TLC pre-coated silica-gel 60 F254 aluminum sheets (0.25 mm, ALUGRAM® SIL G/UV254, Macherey-Nagel, Germany) and RP-18 F254s plates (0.25 mm, Merck, Germany) were used in the detection and separation process. HRESIMS measurements were obtained on a Bruker microTOF mass spectrometer. Compounds were detected by spraying the sheets with 20% v/v H_2SO_4 in EtOH reagent followed by heating at 110 ^{o}C for 1-2 min.

Plant Material. The leaves were collected in June 2009 from organically cultivated plants of six years old from Wadi-Assiut, Assiut, Egypt. The plants cultivation conditions were: temperature 15-37 °C; altitude 68 m asl; latitude: 27° 10' 41.9232" N, longitude: 31° 11' 9.3336" E with a general semi dry and hot climate. The plant was authenticated by Prof. Dr. Abdel-Aziz Fayed, professor of plant taxonomy, Botany Dept., Faculty of Science Assiut,

a voucher specimen (No. 2009SC) was deposited in Pharmacognosy Department Herbarium, Faculty of Pharmacy Assiut University, Assiut Egypt.

Extraction and Isolation. 500 g of the air-dried powdered leaves were extracted by maceration (48 h × 3) with 70% EtOH till complete exhaustion (3L × 3). The alcoholic extract was concentrated and the solvent free residue (47 g, 9.4%) was mixed with 500 mL of distilled H_2O, and subjected to successive solvent fractionation with *n*-hexane, chloroform and ethyl acetate till complete exhaustion in each case to give an *n*-hexane fraction (21 g), chloroform fraction (4 g), ethyl acetate fraction (6 g) and aqueous fraction (14 g). The ethyl acetate fraction was subjected to normal silica gel column using $CHCl_3$–MeOH mixtures in a manner of increasing polarities and the collected fractions were subjected to further chromatographic separation using HPLC Agilent Chromatorex Zorbax SB (C18, 9.4 × 250 mm, 5 μm) using a gradient of 5–80% CH_3CN–H_2O over 40 min to give compound (**3**) (15.8 mg, white powder), (**4**) (11.9 mg, light yellow powder), (**5**) (6.7 mg, light yellow powder) and (**6**) (5.2 mg, light yellow powder).

The aqueous fraction (14 g) was subjected to Diaion-HP_{20} CC using H_2O and MeOH (5 L each). The methanolic elute was concentrated under reduced pressure to yield a fraction (10.6 g). The methanolic fraction was subjected to flash chromatography on silica gel column using $CHCl_3$–MeOH mixtures in a manner of increasing polarities. Ninety fractions (20 mL each) were collected and monitored on (silica gel) TLC using $CHCl_3$–MeOH–H_2O (80:20:2), (70:30:3) and (55:40:5), as well as on RP-C18 using H_2O–MeOH (70:30), (50:50) and (30:70) as solvent systems and 10% v/v H_2SO_4 in EtOH as spraying reagent.

Similar fractions on TLC were combined to yield nine groups. Group (**8**) was subjected to gel filtration chromatography using a Sephadex LH-20 column with $CHCl_3$–MeOH (3:2) followed by HPLC Agilent Chromatorex Eclipse XDB (C3, 9.4 × 250 mm, 5 μm) using a

gradient of 5–30% CH_3CN–H_2O over 40 min to give compound (**1**) (7.1 mg, light yellow amorphous powder) and (**2**) (9.9 mg, light yellow amorphous powder).

*Simmonoside A **(1)***: light yellow amorphous powder; $([\alpha]^{23}_D$ -136.3 (*c* 0.15, MeOH); UV: $\lambda_{max}{}^{MeOH}$: 217, 246 nm; IR (NaCl) ν_{max}: 3454, 3049, 2962, 2247, 1650, 1453, 1245, 1138 cm^{-1}; ^1H, ^{13}C NMR and ^1H-^{13}C HMBC, see Table 1; HRESIMS m/z 649.2215 $[M + Na]^+$ (calcd for $C_{28}H_{38}N_2O_{14}Na$, 649.2221).

*Simmonoside B **(2)***: light yellow amorphous powder; $([\alpha]^{23}_D$ -165.2 (*c* 0.15, MeOH); UV: $\lambda_{max}{}^{MeOH}$: 220, 231 nm; IR (NaCl) ν_{max}: 3449, 2947, 2223, 1641, 1430, 1261, 1115 cm^{-1}; ^1H, ^{13}C NMR and ^1H-^{13}C HMBC, see Table 2; HRESIMS m/z 649.2227 $[M + Na]^+$ (calcd for $C_{28}H_{38}N_2O_{14}Na$, 649.2221).

Cytotoxicity Assays. The *in vitro* cancer growth inhibitory activity of the isolated compounds was determined using MTT colorimetric assay against A-549 (human lung carcinoma) and SGC-7901 (human gastric cancer) cell lines. A-549 obtained from ATCC (American Type Culture Collection) whilst (SGC-7901) was obtained from the Cell Bank of Type Culture Collection of Chinese Academy of Sciences, Shanghai Institute of Cell Biology. A-549 was cultured in Dulbecco's modified Eagle's medium (DMEM) with 4.5 g/l glucose (Sigma–Aldrich), supplemented with 5% fetal bovine serum (Gibco) and 1% of a penicillin–streptomycin mixture (10.000 UI/mL and 10 mg/mL, respectively). Cells were maintained at 37 ^oC in 5% CO_2 and 98% humidity. SGC-7901 cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 IU/mL penicillin and 100 mg/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO_2. Cells at the logarithmic phase were used for experiments. The cytotoxicity was determined based on the cell viability and measured by the colorimetric MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] reduction assay. Briefly, A-549 and SGC-7901 cells were cultured on a 96-well plate each at a density of 2×10^4 cells/well per 100 µL. After 24

hours, drugs predissolved in DMSO at different concentration ranging from 0.01 to 100 μM were added to the culture (the final concentration of DMSO was 0.1%) and three replicate wells were used for each of the concentrations. Untreated and blank groups were set as controls. After 48-hour exposure, the MTT solution (5 mg/mL in phosphate buffered saline, PBS) was added to the wells and the plates were incubated for an additional 4 h in a CO_2 incubator at 37 ^{o}C, then the supernatant was discarded, each well was added 150 µL of dimethylsulfoxide (DMSO). The absorbance was measured at 490 nm in Bio-TekEXL808 microplate reader (Bio-Tek, Winooski, VT, USA).[15-17]

COX-2 Inhibitory Assay. The activity of the test compounds (final concentrations of 100, 10, 1, 0.1 and 0.01 µM) on COX-2 was determined by measuring prostaglandin E2 (PGE2) using a COX Inhibitor Screening Kit (Catalog No 560131, Cayman Chemicals, Ann Arbor Michigan USA) according to Bertanha et al. method.[18] Reaction mixtures (1.15 mL) containing the reaction buffer (950 µL), heme (10 µL), COX-2 (10 µL) and sample/control (20 µL) were incubated at 37 °C in a water bath for 15 min. The reaction was initiated by addition of arachidonic acid (10 µL) at a final concentration of 100 µM. After 2 min incubation, the reactions were stopped by addition of 1 M HCl (50 µL), followed by saturated solution of stannous chloride (100 µL). Then, prostaglandins (PGs) were quantified by means of the ELISA method. The contents of the reaction tubes were diluted and transferred to a 96-well plate coated with a mouse anti-rabbit IgG, followed by addition of the PG screening acetylcholinesterase tracer and the PG screening antiserum. Plates were incubated in an orbital shaker for 18 h, at room temperature. The reaction mixtures were removed, and the wells were washed five times with a buffer containing 0.05 % Tween 20. Acetylthiocholine and 5,5'-dithio-bis-2- nitro-benzoic acid known as Ellman's reagent (200 µL) was then added to each well, and the plate was incubated in an orbital shaker for 60 min, at room temperature, until the control wells yielded an optical density lying between 0.3–0.8 at 415

nm. A standard curve with PG was generated from the same plate, which was used to quantify the PG levels produced in the presence of the samples. The compound DuP697 (Cayman Chemicals) was used to standardize the assay for COX-2 and indomethacin was employed as positive control. All determinations were performed in triplicate. Regression analysis (probit analysis, SPSS 16.0) was employed for the calculation of IC_{50} values.[19]

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Table S1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR spectroscopic data of compounds (**3-6**) in DMSO-d_6.

No.	**3**		**4**		**5**		**6**	
	^{13}C	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**	**^{13}C**	**^{1}H**
1	73.3 CH	4.49 (1H, m)	74.0 CH	4.79 (1H, t, 4.5)	73.5 CH	4.68 (1H, t, 5.3)	73.7 CH	4.68 (1H, m)
2	158.4 C	-	164.8 C	-	164.9 C	-	164.7 C	-
3	122.5 CH	6.44 (1H, dd, 1.9, 10.2)	68.9 CH	4.52 (1H, d, 8.0)	71.6 CH	4.32 (1H, d, 7.5)	70.0 CH	4.46 (1H, d, 7.5)
4	144.4 CH	6.25 (1H, dd, 1.3, 10.2)	85.4 CH	2.94 (1H, dd, 8.0, 3.0)	73.2 CH	3.48 (1H, m)	82.9 CH	3.19 (1H, m)
5	65.7 CH	4.37 (1H, m)	65.1 CH	4.08 (1H, m)	77.2 CH	3.56 (1H, m)	75.1 CH	3.72 (1H, m)
6	39.0 CH_2	a. 2.43 (1H, m) b. 1.45 (1H, q, 12.3)	33.3 CH_2	a. 2.19 (1H, m) b. 1.68 (1H, m)	30.6 CH_2	a. 2.10 (1H, m) b. 1.80 (1H, m)	31.0 CH_2	a. 2.09 (1H, m) b. 1.81 (1H, m)
7	92.0 CH	5.72 (1H, brs)	93.9 CH	5.70 (1H, s)	93.7 CH	5.61 (1H, s)	94.6 CH	5.67 (1H, s)
8	117.4 C	-	116.6 C	-	116.8 C	-	117.0 C	-
CH_3O-4	-	-	57.5 CH_3	3.35 (3H, s)	-	-	57.8 CH_3	3.33 (3H, s)
CH_3O-5	-	-	-	-	56.6 CH_3	3.30 (3H, s)	56.9 CH_3	3.28 (3H, s)
1′	101.4 CH	4.34 (1H, d, 8.1)	100.7 CH	4.34 (1H, d, 8.0)	101.2 CH	4.28 (1H, d, 8.0)	101.6 CH	4.27 (1H, d, 7.5)
2′	73.3 CH	3.00 (1H, t, 8.4)	73.4 CH	2.98 (1H, t, 7.9)	73.0 CH	3.03 (1H, m)	73.3 CH	3.03 (1H, m)
3′	76.7 CH	3.17 (1H, m)	76.5 CH	3.14 (1H, m)	76.6 CH	3.13 (1H, m)	76.9 CH	3.14 (1H, m)
4′	70.0 CH	3.07 (1H, m)	69.9 CH	3.09 (1H, m)	69.8 CH	3.05 (1H, m)	70.1 CH	3.06 (1H, m)
5′	77.0 CH	3.12 (1H, m)	76.8 CH	3.11 (1H, m)	76.8 CH	3.07 (1H, m)	77.0 CH	3.06 (1H, m)
6′	61.1 CH_2	a. 3.64 (1H, d, 11.0) b. 3.44 (1H, m)	61.0 CH_2	a. 3.63 (1H, m) b. 3.47 (1H, m)	60.8 CH_2	a. 3.64 (1H, d, 11.9) b. 3.44 (1H, m)	61.2 CH_2	a. 3.63 (1H, m) b. 3.43 (1H, m)
OH-3	-	-	-	5.66 (1H, brs)	-	5.60 (1H, brs)	-	5.67 (1H, brs)
OH-4	-	-	-	-	-	4.94 (1H, brs)	-	-
OH-5	-	5.37 (1H, brs)	-	4.68 (1H, brs)	-	-	-	-
OH-2′	-	5.14 (1H, brs)	-	5.02 (1H, brs)	-	4.75 (1H, brs)	-	4.78 (1H, brs)
OH-3′	-	5.02 (1H, brs)	-	4.97 (1H, brs)	-	4.98 (1H, brs)	-	4.96 (1H, brs)
OH-4′	-	4.98 (1H, brs)	-	4.93 (1H, brs)	-	4.93 (1H, brs)	-	4.91 (1H, brs)
OH-6′	-	4.49 (1H, m)	-	4.24 (1H, t, 5.3)	-	4.24 (1H, m)	-	4.24 (1H, m)

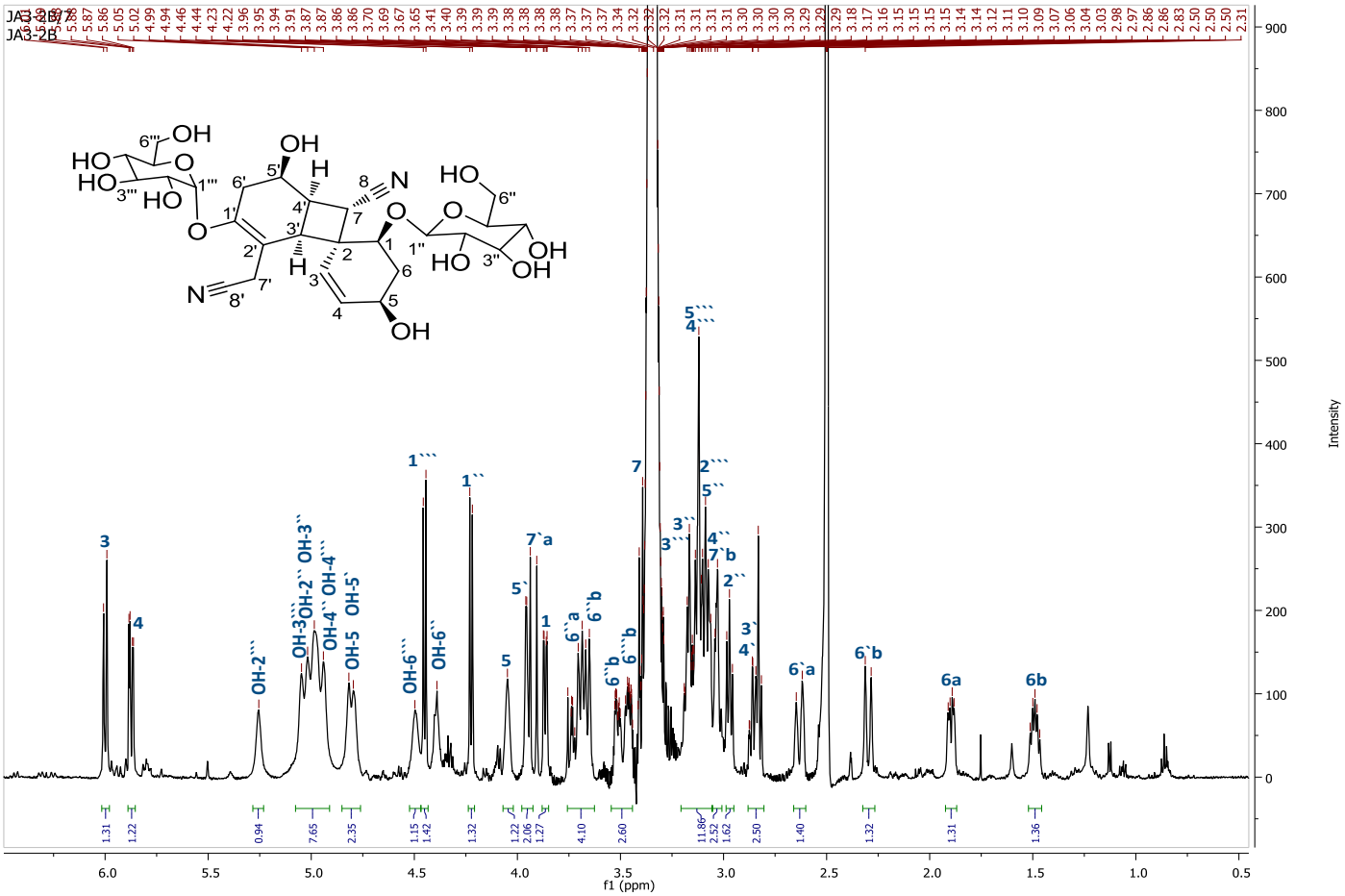


Figure S1. ^{1}H NMR spectrum of compound (**1**) (600 MHz, DMSO-d_6)

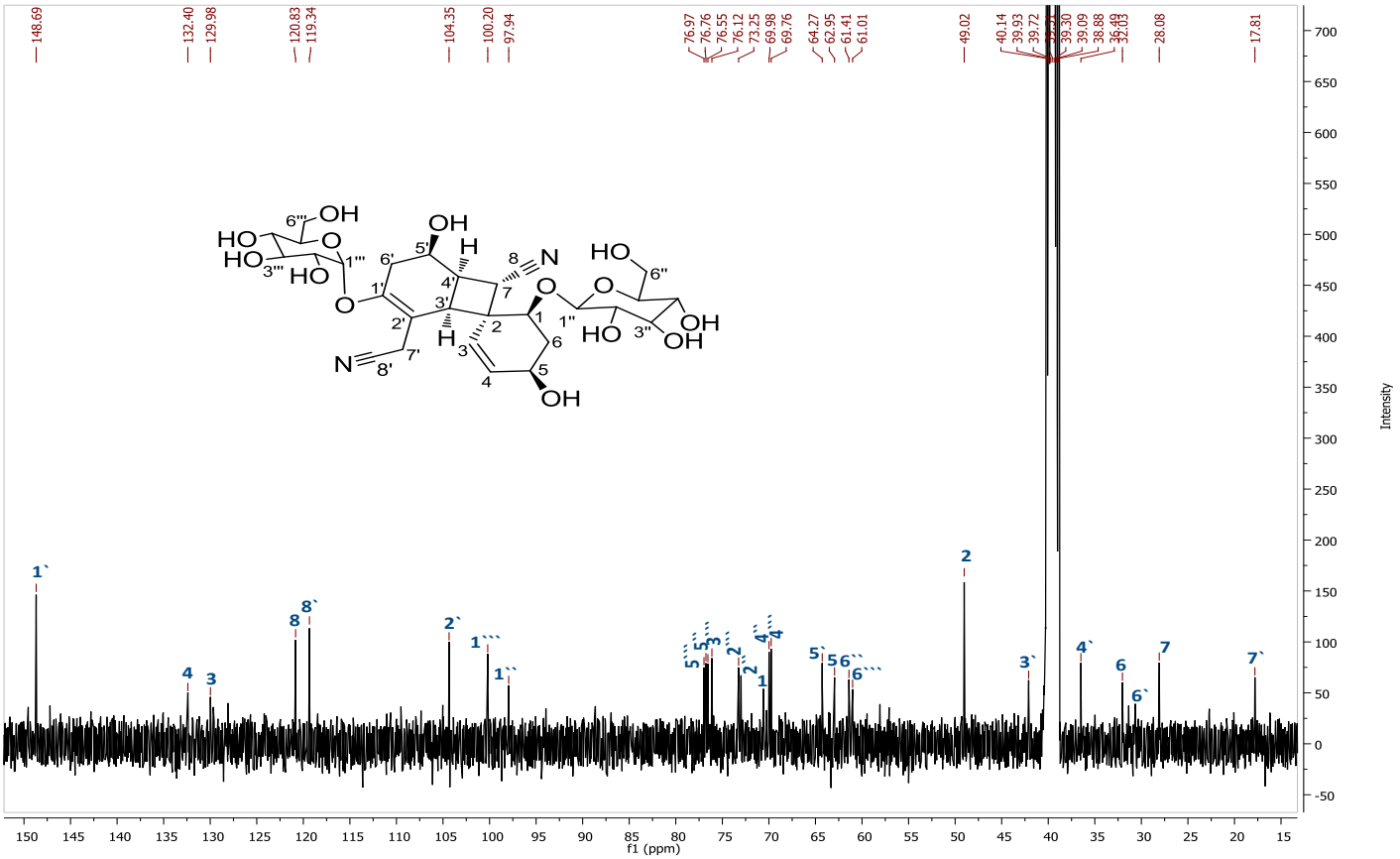


Figure S2. ^{13}C NMR spectrum of compound (**1**) (150 MHz, DMSO-d_6)

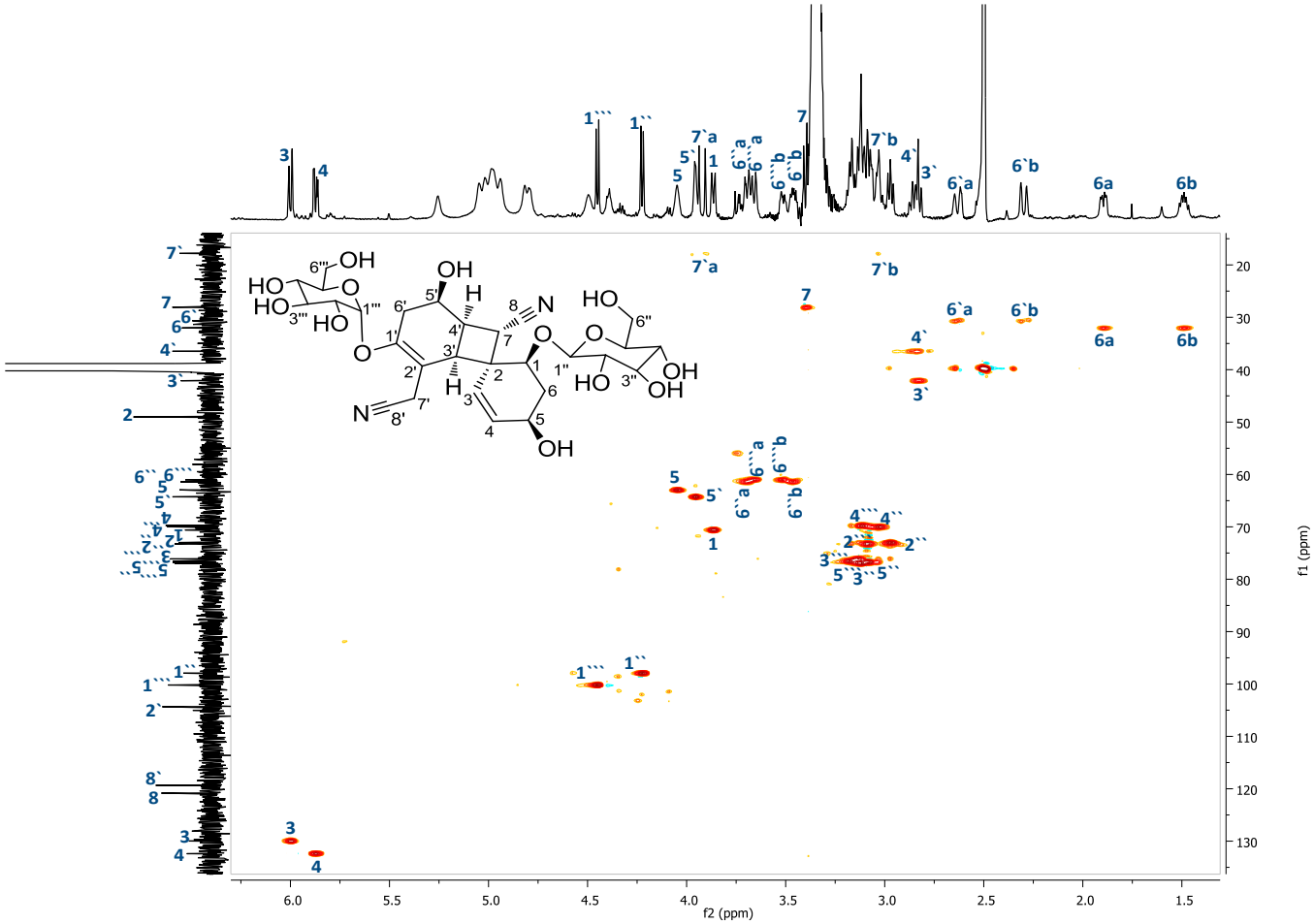


Figure S3. ^{1}H-^{13}C HSQC spectrum of compound (**1**) (600 MHz, DMSO-d_6)

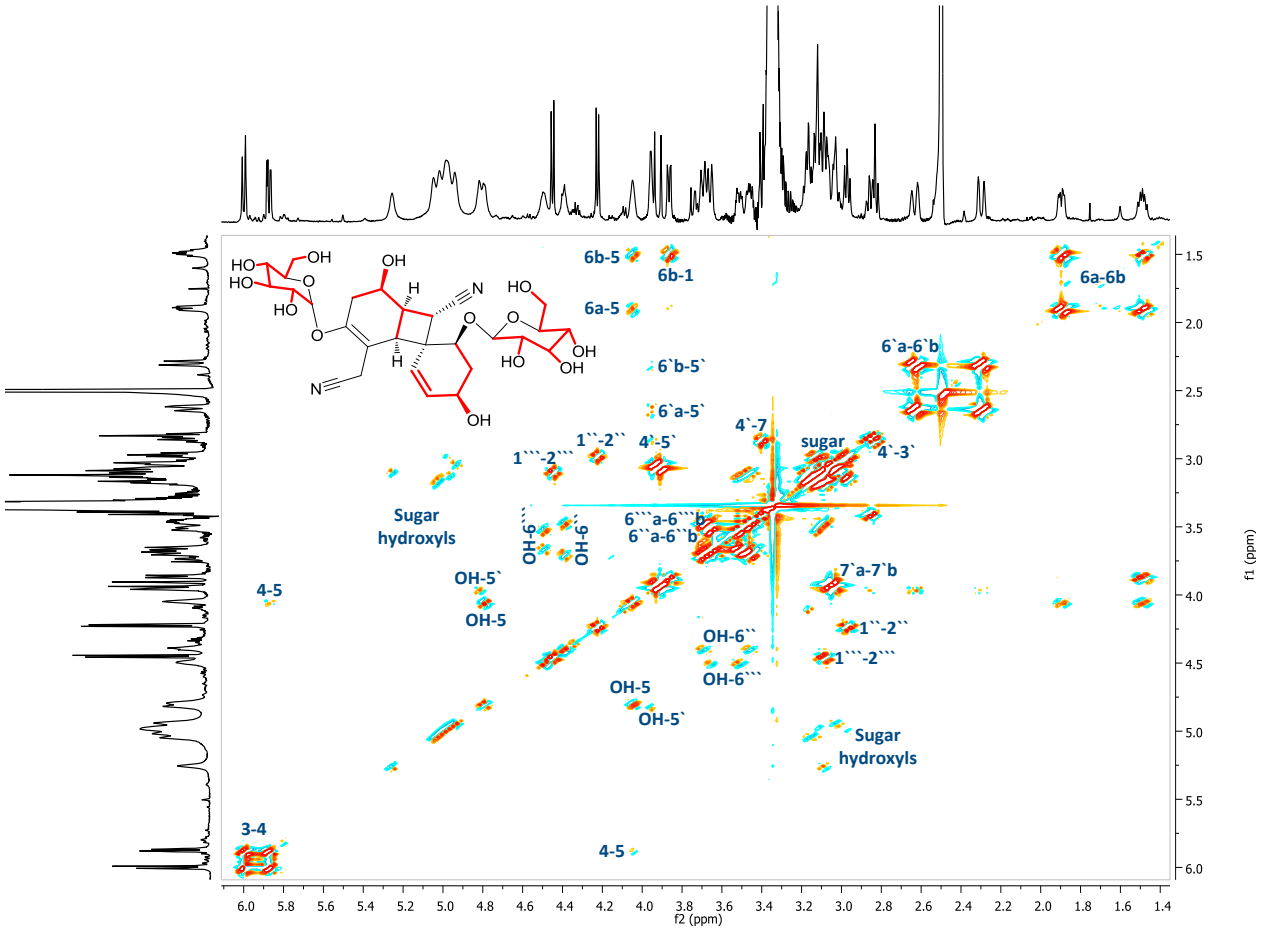


Figure S4. ^{1}H-^{1}H COSY spectrum of compound (**1**) (600 MHz, DMSO-d_6)

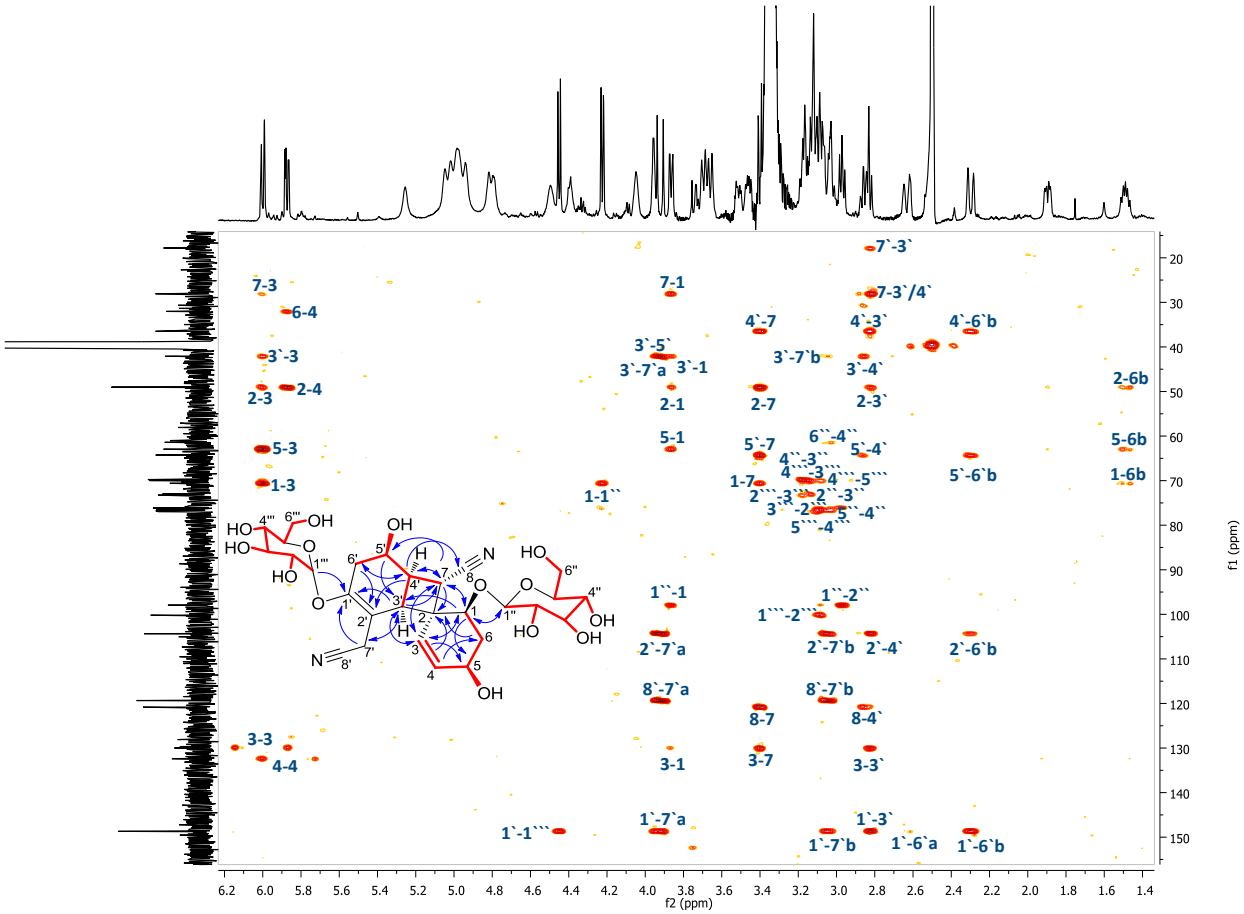


Figure S5. ^{1}H-^{13}C HMBC spectrum of compound (**1**) (600 MHz, DMSO-d_6)

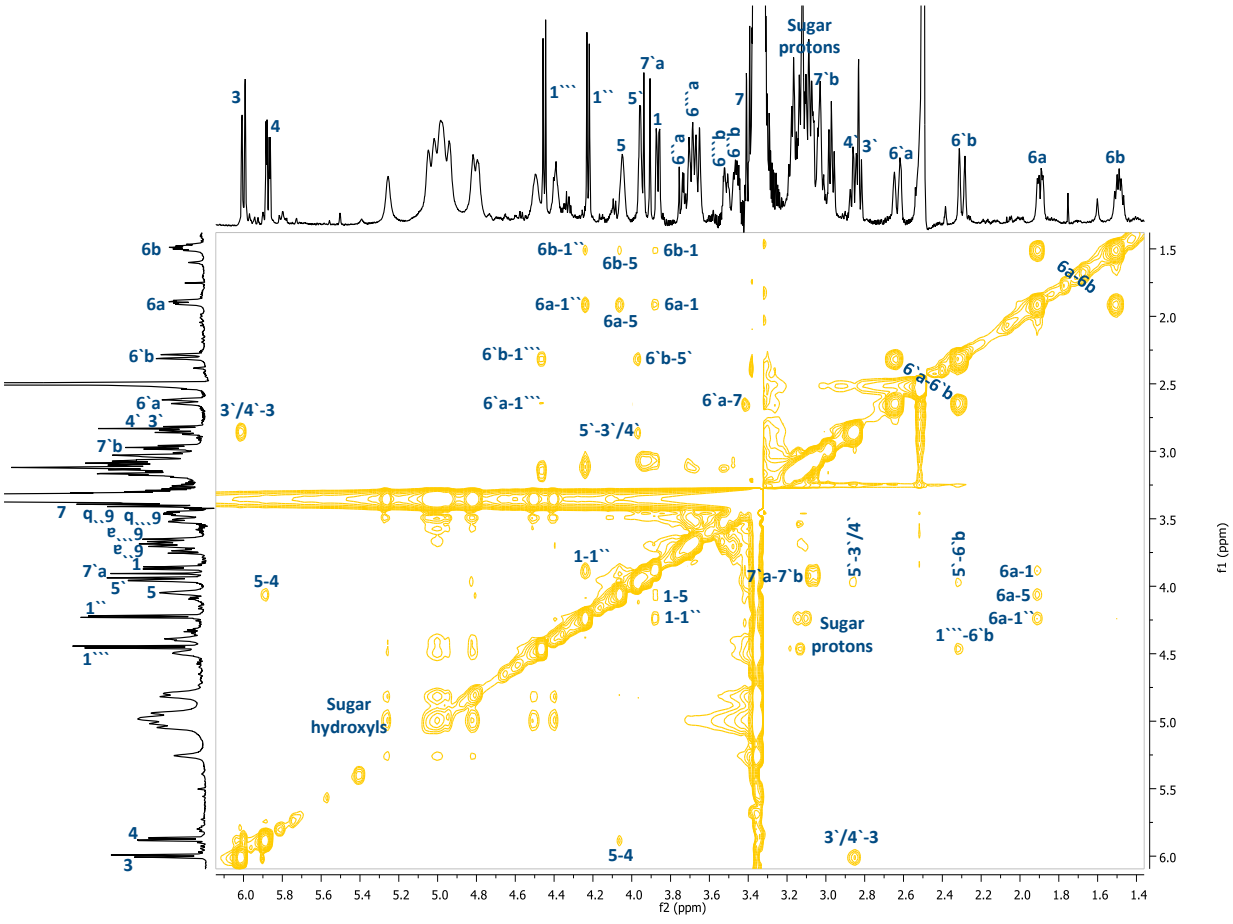


Figure S6. ^{1}H-^{1}H NOESY spectrum of compound (**1**) (600 MHz, DMSO-d_6)

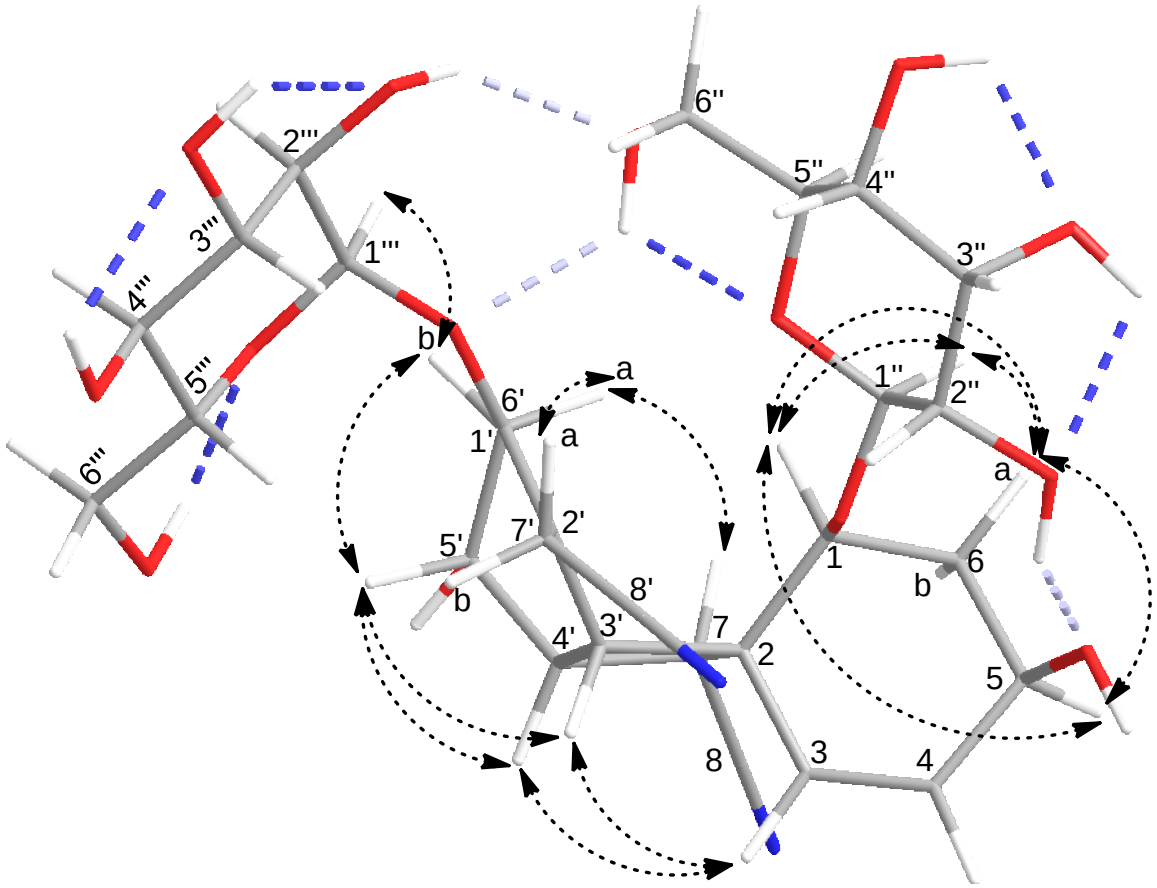


Figure S7. Key NOESY correlations and global energy minimum for compound **1**.

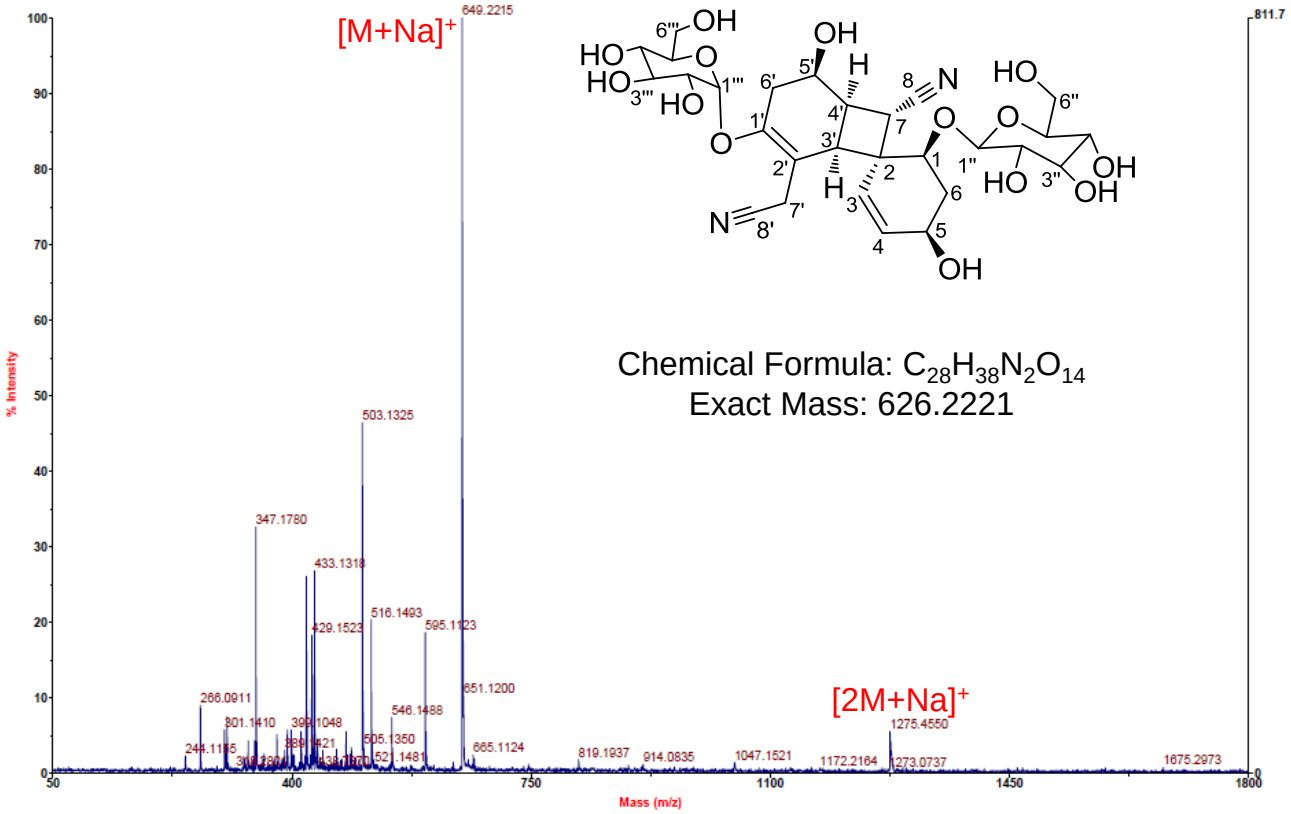


Figure S8. HRESIMS of compound (**1**)

Table S2. ^{1}H and ^{13}C NMR (in DMSO-d_6) Assignments and HMBC Correlations of **1**.

No.	δ_C, mult.	δ_H, mult (*J* in Hz)	HMBC (H→C#)
1	70.6, CH	3.87, dd (*2.1, 8.4*)	7,3′,2,5,1″,3
2	49.0, C		
3	130.0, CH	6.00, d (*10.0*)	7,3′,2,5,1,3
4	132.4, CH	5.87, dd (*3.3*, *10.0*)	6,2,4
5	63.0, CH	4.05, m	
6	32.0, CH_2	a. 1.89, m	
		b. 1.49, m	2,5,1
7	28.1, CH	3.40, m	4′,2,5′,8,3
8	120.8, C		
Sugar I	*Glucose*	*Glucose*	*Glucose*
1″	97.9, CH	4.23, d (*7.5*)	1
2″	73.0, CH	2.97, m	1″
3″	76.1, CH	3.13, m	4″,2″
4″	70.0, CH	3.03, m	6″,5″
5″	76.8, CH	3.07, m	
6″	61.4, CH_2	a. 3.69, m	
		b. 3.46, m	
OH-5		4.80, brs	
OH-2″		4.99, brs	
OH-3″		5.02, brs	
OH-4″		4.94, brs	
OH-6″		4.39, brs	
1′	148.7, C		
2′	104.4, C		
3′	42.1, CH	2.83, m	7′,7,4′,2,3,1′
4′	36.5, CH	2.84, m	7,3′,5′,2′,8
5′	64.3, CH	3.96, m	3′
6′	30.6, CH_2	a. 2.63, m	1′
		b. 2.30, m	1′,2′,5′,4′
7′	17.8, CH_2	a. 3.92, d (*18.0*)	1′,2′,3′,8′
		b. 3.05, d (*18.0*)	1′,2′,8′
8′	119.3, C		
Sugar II	*Glucose*	*Glucose*	*Glucose*
1‴	100.2, CH	4.45, d (*7.5*)	1′
2‴	73.3, CH	3.09, m	1‴,3‴
3‴	76.6, CH	3.17, m	2‴,4‴
4‴	69.8, CH	3.11, m	5‴
5‴	77.0, CH	3.11, m	4‴
6‴	61.0, CH_2	a. 3.66, m	
		b. 3.52, m	
OH-5′		4.82, brs	
OH-2‴		5.25, brs	
OH-3‴		5.05, brs	
OH-4‴		4.98, brs	
OH-6‴		4.51, brs	

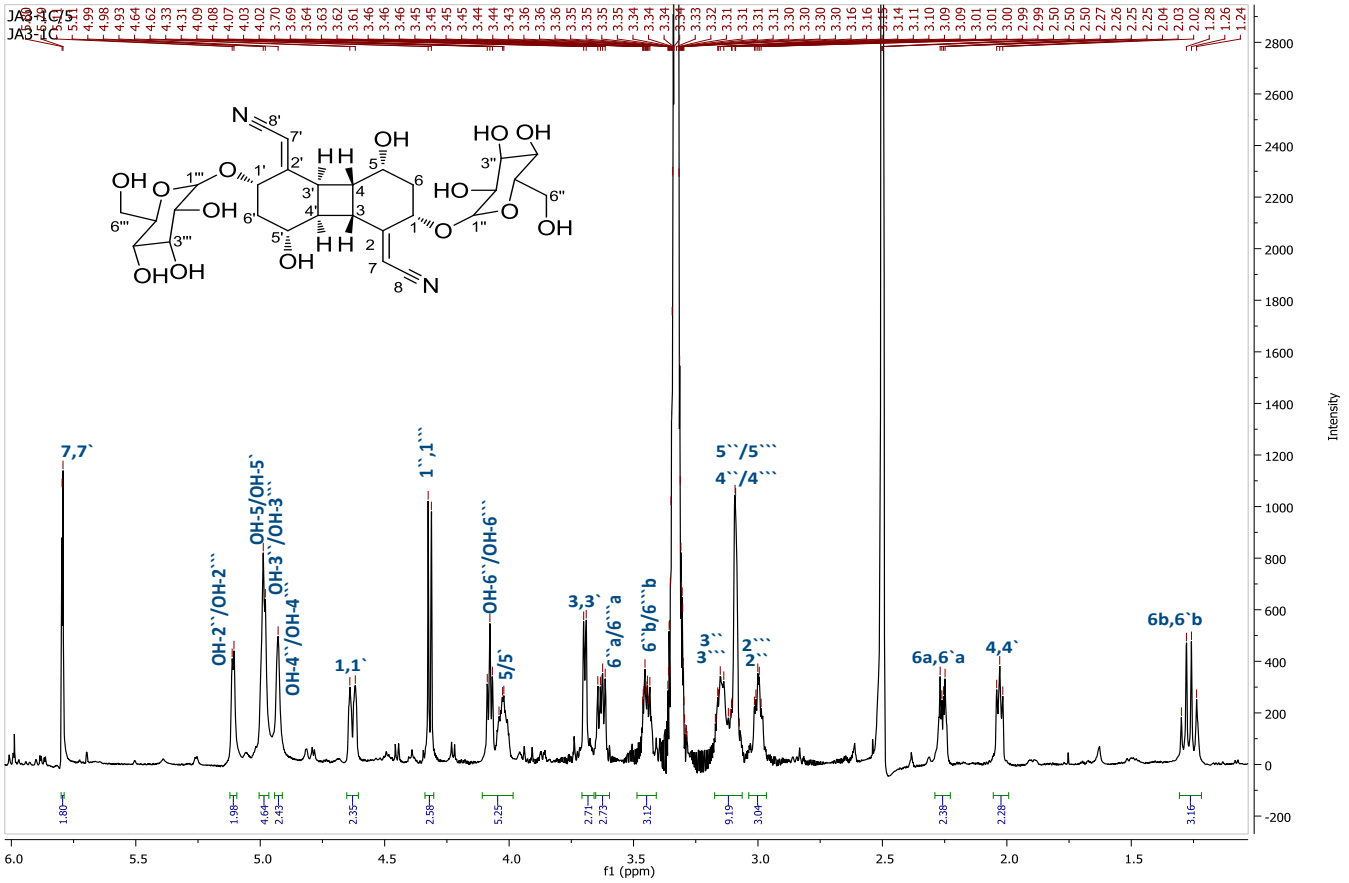


Figure S9. ^{1}H NMR spectrum of compound (**2**) (600 MHz, DMSO-d_6)

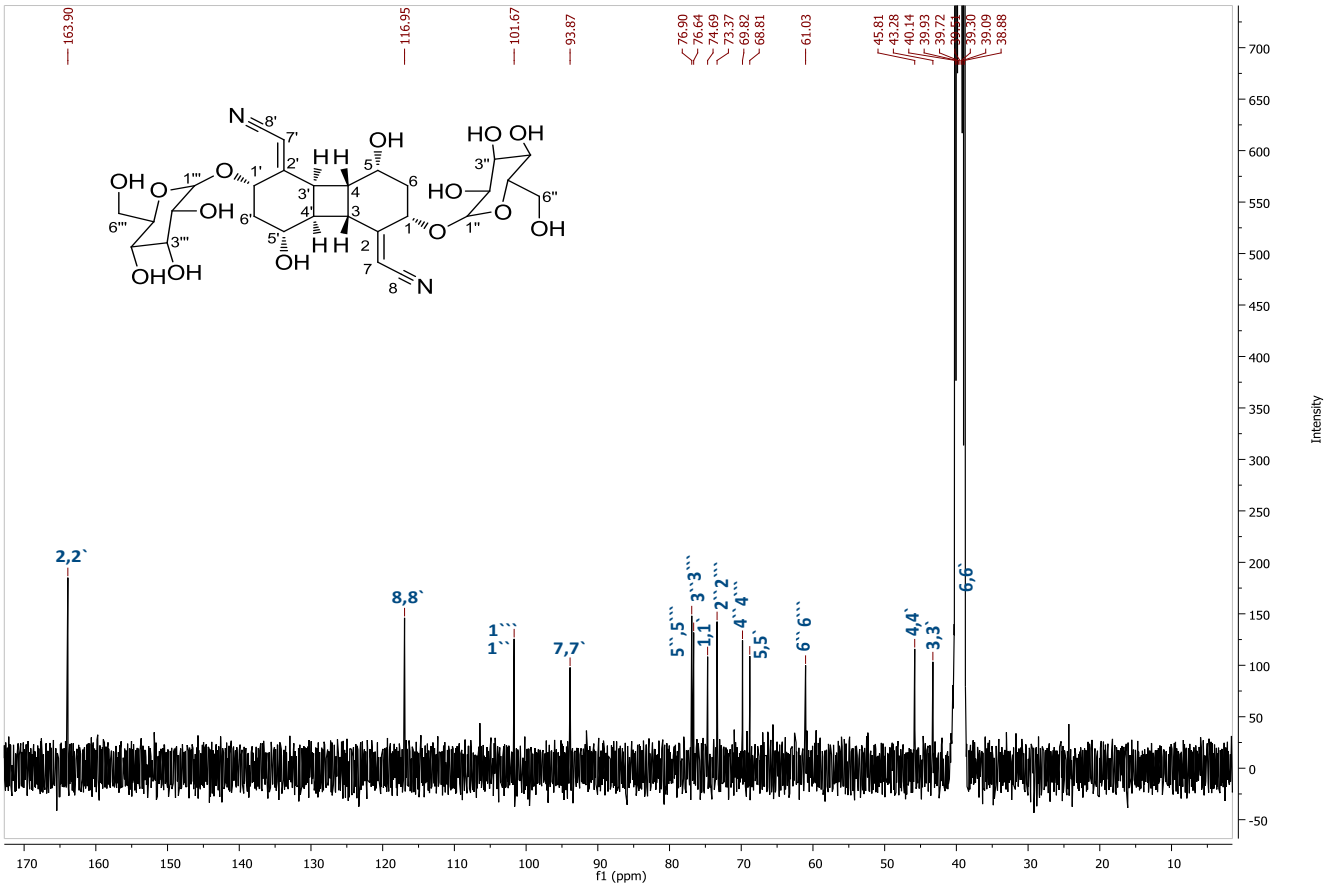


Figure S10. ^{13}C NMR spectrum of compound (**2**) (150 MHz, DMSO-d_6)

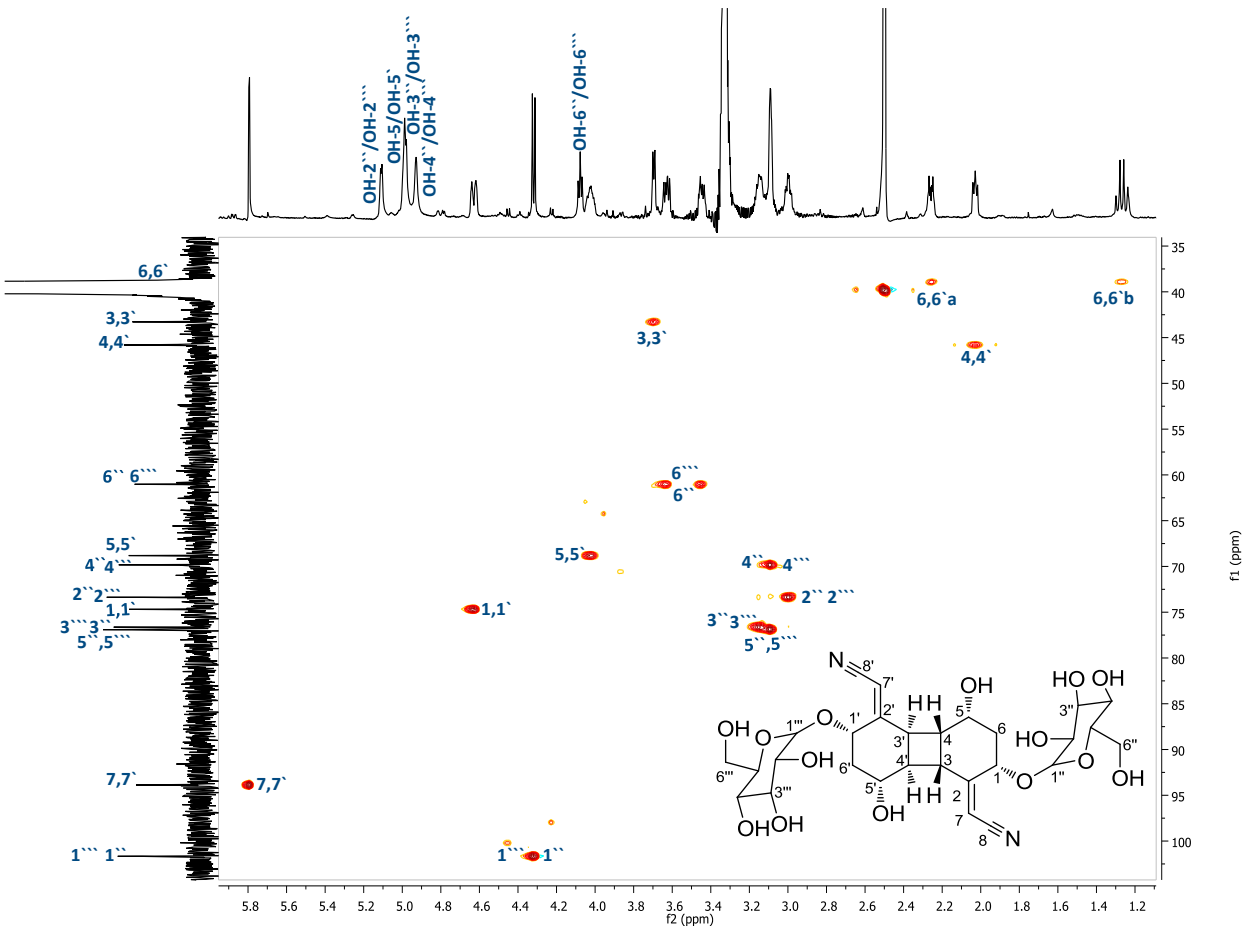


Figure S11. ^{1}H-^{13}C HSQC spectrum of compound (**2**) (600 MHz, DMSO-d_6)

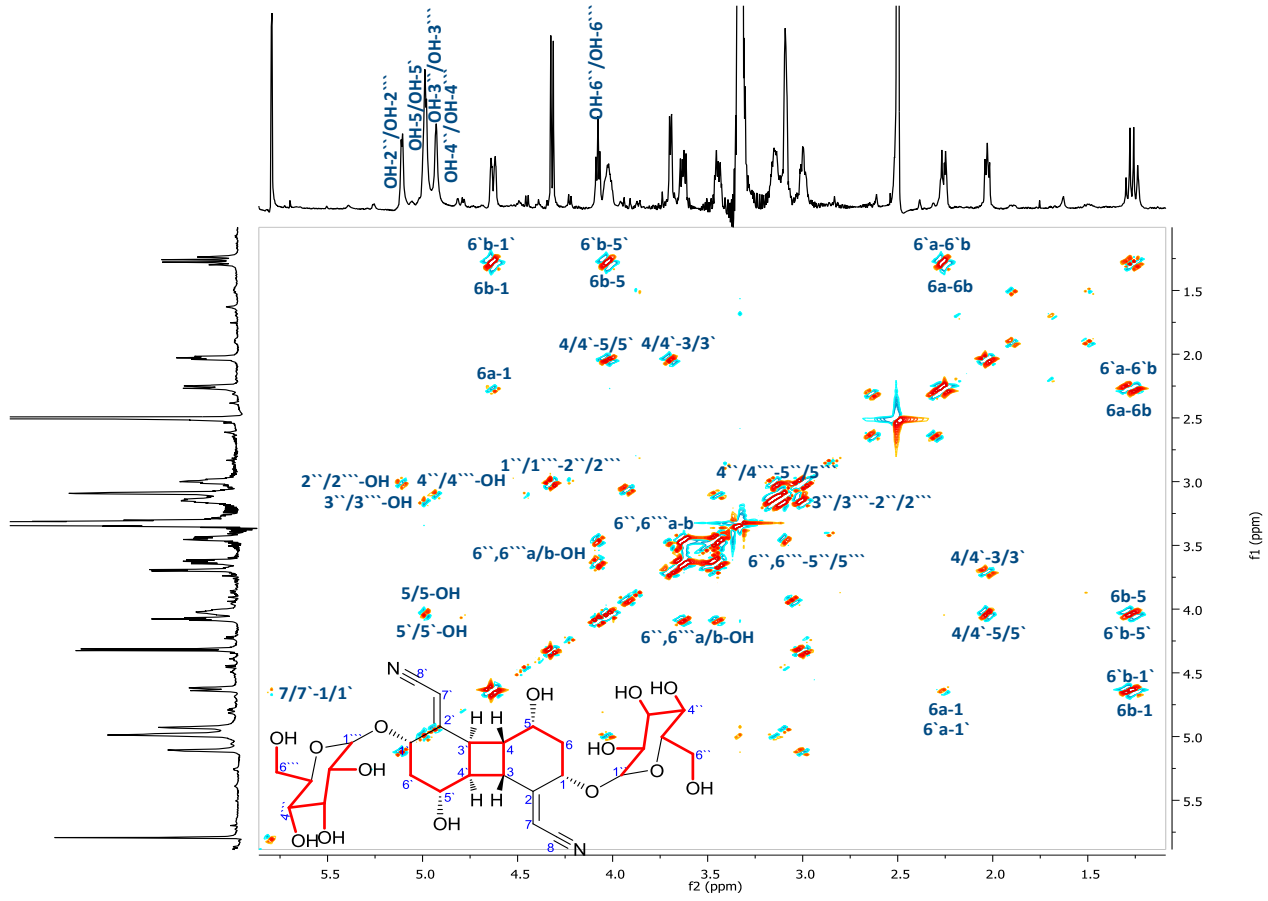


Figure S12. ^{1}H-^{1}H COSY spectrum of compound (**2**) (600 MHz, DMSO-d_6)

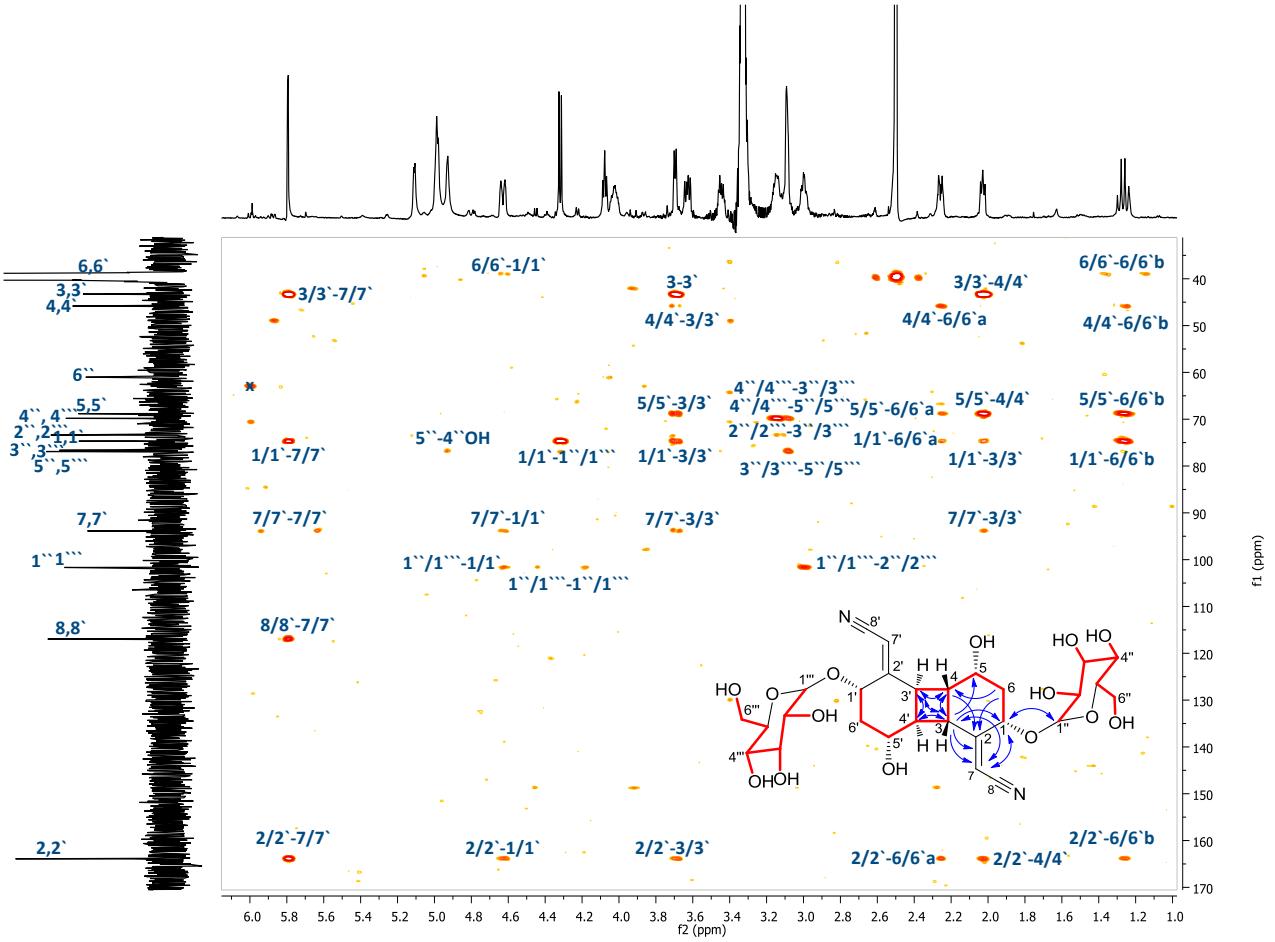


Figure S13. ^{1}H-^{13}C HMBC spectrum of compound (**2**) (600 MHz, DMSO-d_6)

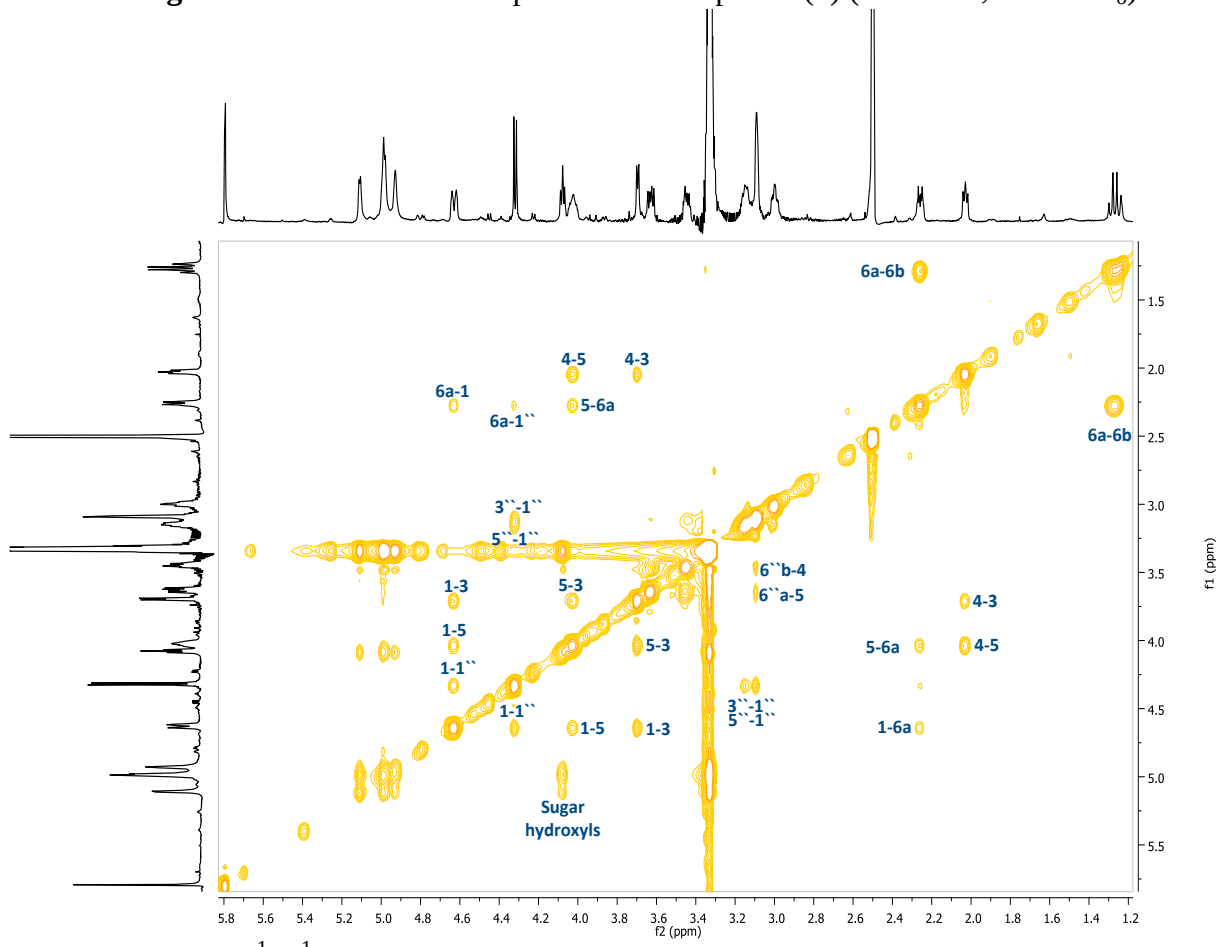


Figure S14. ^{1}H-^{1}H NOESY spectrum of compound (**2**) (600 MHz, DMSO-d_6)

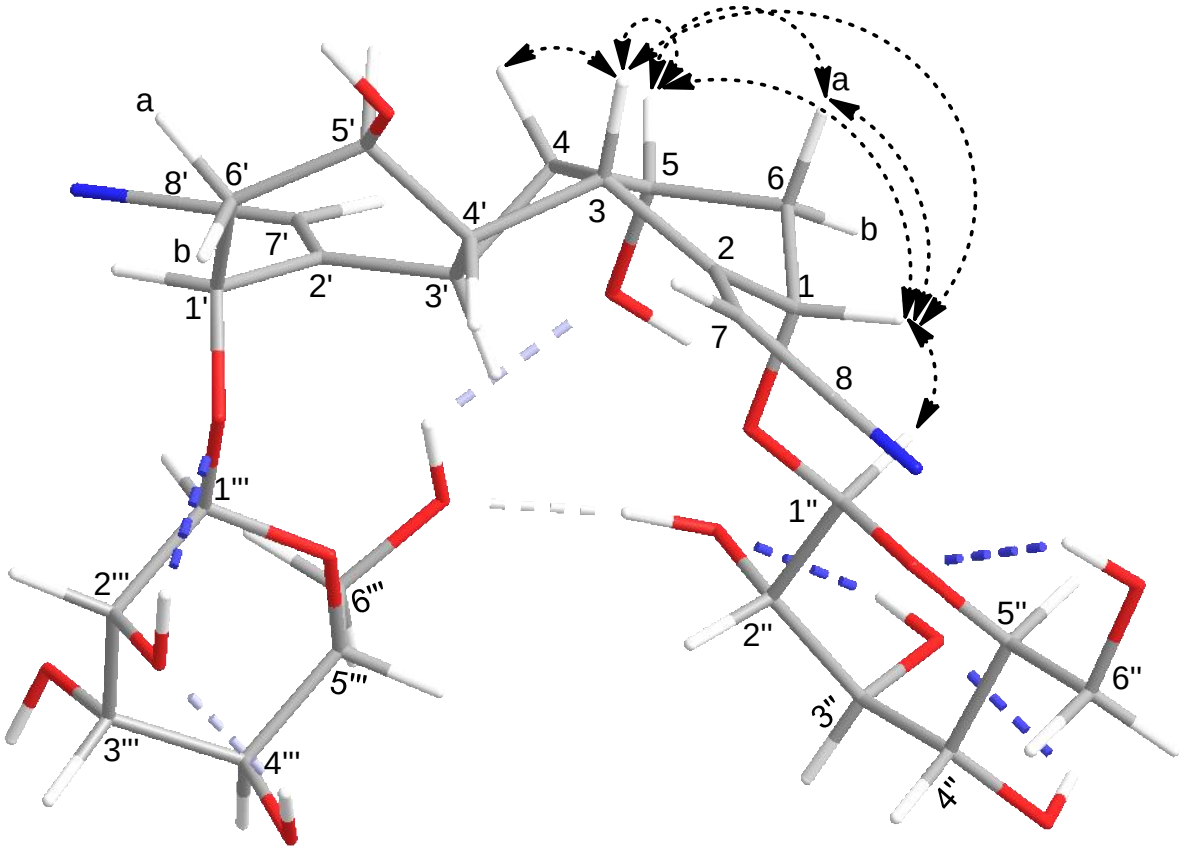


Figure S15. Key NOESY correlations and global energy minimum for compound **2**.

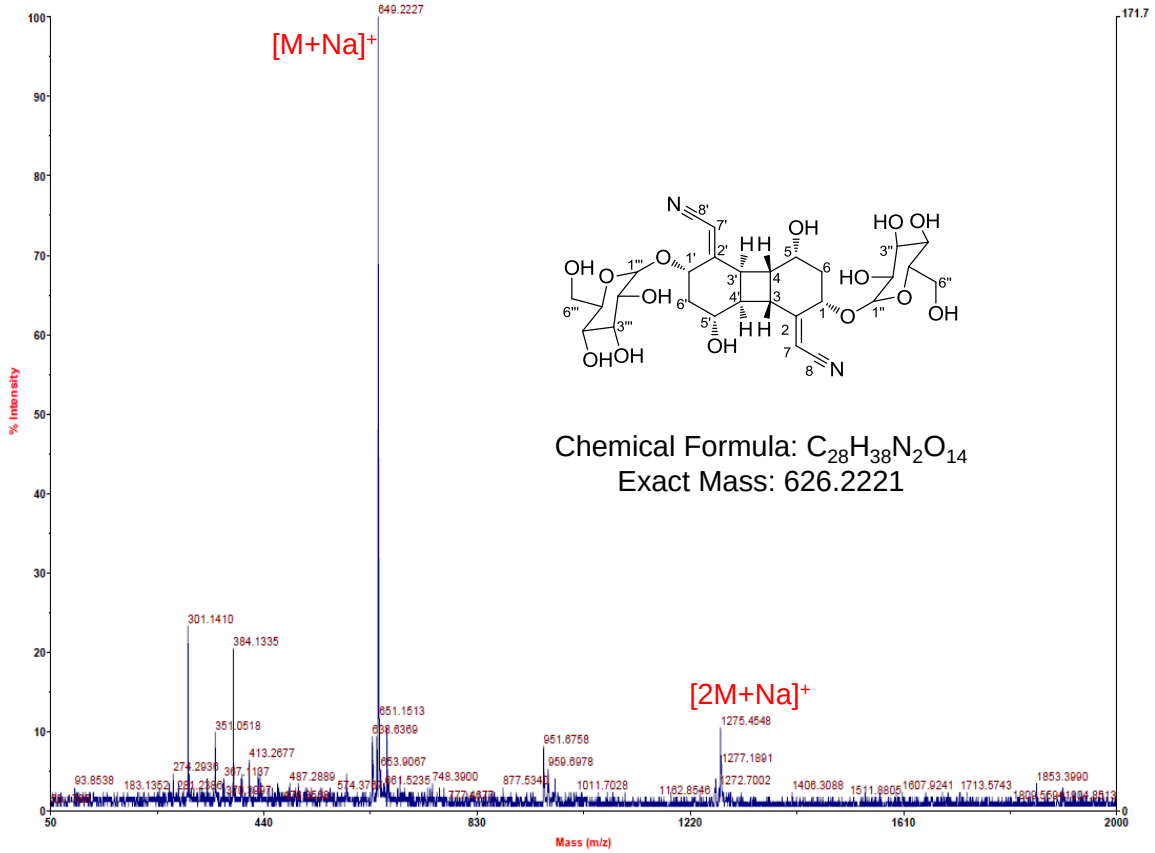
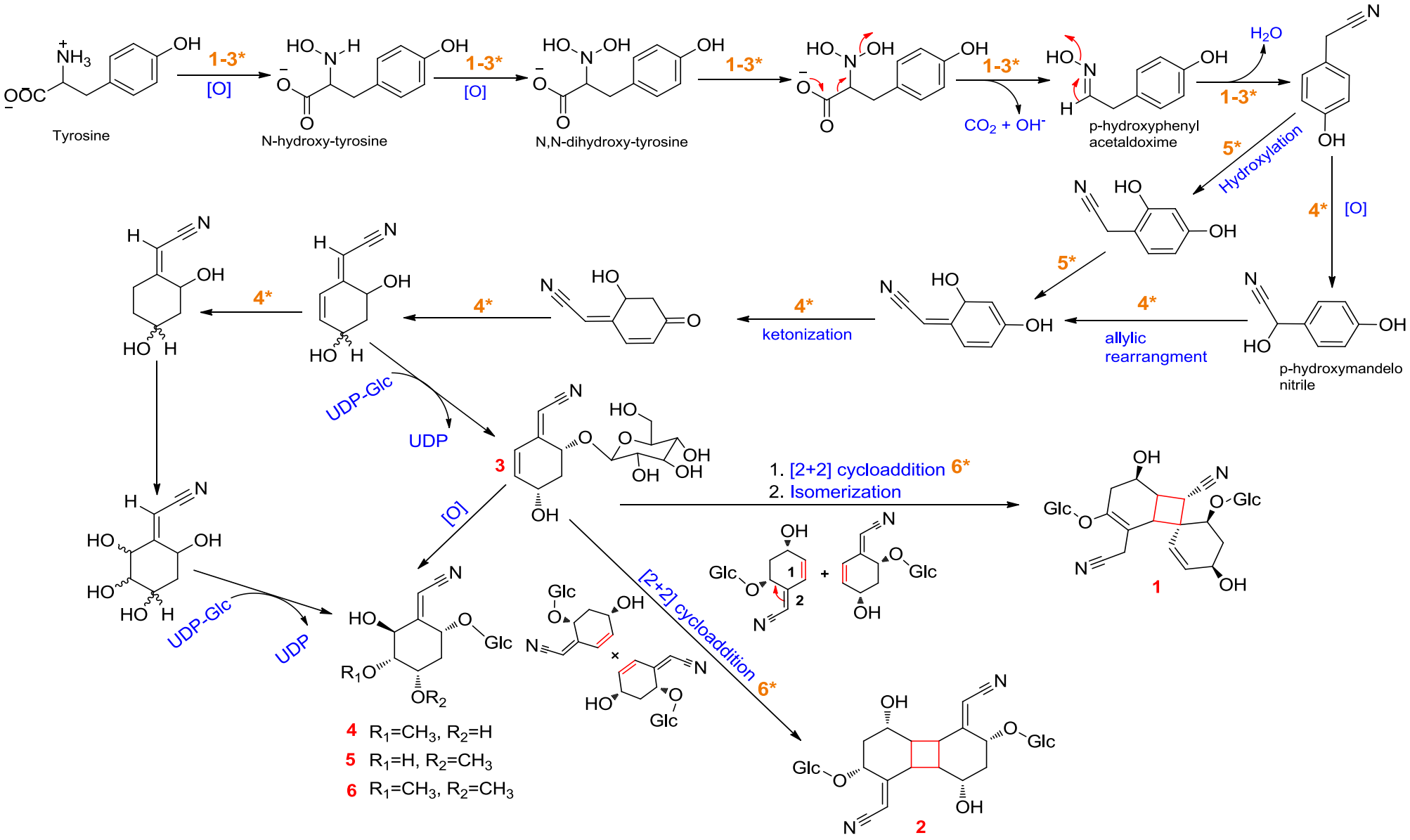


Figure S16. HRESIMS of compound (**2**)

Table S3. ^{1}H and ^{13}C NMR (in DMSO-d_6) Assignments and HMBC Correlations of **2**.

No.	δ_C, mult.	δ_H, mult (*J* in Hz)	HMBC (H→C#)
1, 1′	74.7, CH	4.63, m	2,6,7,1″
2, 2′	163.9, C		
3, 3′	43.3, CH	3.70, d (*6.4*)	1,2,4,5,7,3′,4′
4, 4′	45.8, CH	2.02, m	2,3,5
5, 5′	68.8, CH	4.02, m	
6, 6′	39.1, CH_2	a. 2.25, m	1,2,4,5
		b. 1.27, m	1,2,4,5,6
7, 7′	93.9, CH	5.79, d (*2.2*)	1,2,3,7,8
8, 8′	117.0, C		
Sugar I, II	*Glucose*	*Glucose*	*Glucose*
1″, 1‴	101.7, CH	4.32, d (*7.9*)	1,1″
2″, 2‴	73.4, CH	3.00, m	1″
3″, 3‴	76.6, CH	3.14, m	2″,4″
4″, 4‴	69.8, CH	3.09, m	
5″, 5‴	76.9, CH	3.09, m	3″,4″
6″, 6‴	61.0, CH_2	a. 3.63, dd (*7.0, 11.6*)	
		b. 3.44, m	
OH-5, 5′		4.99, brs	
OH-2″, 2‴		5.11, brs	
OH-3″, 3‴		4.99, brs	
OH-4″, 4‴		4.93, brs	5″
OH-6″, 6‴		4.07, brs	



Scheme S1. Proposed biogenetic scheme for isolated compounds (**1-6**).

***Proposed biogenetic scheme references**

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