Supporting Information

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Cyclocarioside Z14, A New Dammarane Triterpenoid Glycoside from the Leaves of *Cyclocarya paliurus* with Cytotoxicity

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Table of Contents	Page

General experimental procedures

Optical rotation was measured on JASCO model 1020 polarimeter (Horiba, Tokyo, Japan) at 25°C. Silica gel (200-300 or 80-100 mesh; Qingdao Peremanent Sea Silica Ltd., Qingdao, China), polyamide (80-100 or 30-60 mesh, Taizhou Luqiao Sijia Biochemical Plastics Factory, Taizhou, China), and C18 reverse-phased silica gel (40-75 μm, Fuji, Kasugai, Japan) were used for column chromatography (CC) separations. Analytical HPLC experiments were conducted with a YMC Pack ODS-A column (5μm, 250 mm × 4.6 mm i.d.; Tokyo, Japan) in Agilent 1100 (Agilent Technologies, Ltd.) equipped with a diode array detector (DAD) under reversed-phase. UV spectra were recorded on Waters Acquity UPLC equipped 2998 PDA Detector (America). NMR spectral data were obtained on Bruker AV-500 MHz spectrometer (Bruker, Karlsruhe, Germany) using CD_3OD as a solvent and tetramethylsilane (TMS) as an internal standard at room temperature. HR-ESI-MS were acquired on LC-LTQ Orbitrap Velos Pro ETD (Thermo Fisher, MA, USA) in positive ion mode. Gas Chromatography-Mass Spectrometer (GC-MS) experiment was performed on GCMS-QP2010 Ultra (SHIMADZU, Hongkong, China). Unless stated otherwise, all the chemical solvents were analytical grade (Cologne Chemical Co., Ltd., Chengdu, China).

Cytotoxicity evaluation

All isolated compounds were determinedagainst seven human cancer cell lines, including Du145, PC-3, MCF-7, SKVO3, NCI-H1975, PC-9 and HepG2. The cells were cultured in complete medium at 37°Cin a humidified atmosphere with 5% CO_2, and supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution. According to the MTT assay which was reported in our previous study [15], the cytotoxicity assay was performed with the positive control of staurosporine (STS) in 96-well microplates.

Acid hydrolysis

Compound **1** (1 mg) was hydrolyzed in 2.0 M HCl (2 mL) under reflux in the hot oil bath (100°C, 2 h). The reaction mixture was neutralized with Na_2CO_3 and extracted thrice with chloroform ($CHCl_3$). The aqueous layer was concentrated and dried to yield the sugar moiety. The mixture was dissolved in pyridine (1 mL) followed by L-cysteine methyl ester hydrochloride (2 mg) and heated in the hot oil bath (60°C, 1 h). After the reaction completed, trimethylsilylimidazole (1 mL) was added and heated in the hot water bath (60°C, 30 min). The sample was filtered by 0.22 μM filter membrane and analyzed by GC-MS: Column, Rxi-5Sil MS (0.25 μm × 30.0 mm, 0.32 mm); front inlet 300°C, column 150°C-300°C at 15°C/min. The sugar units of compound **1** were identified by the comparison of the retention times with authentic standard L-arabinofuranose and D-quinovose treated in the same manner under the same conditions.

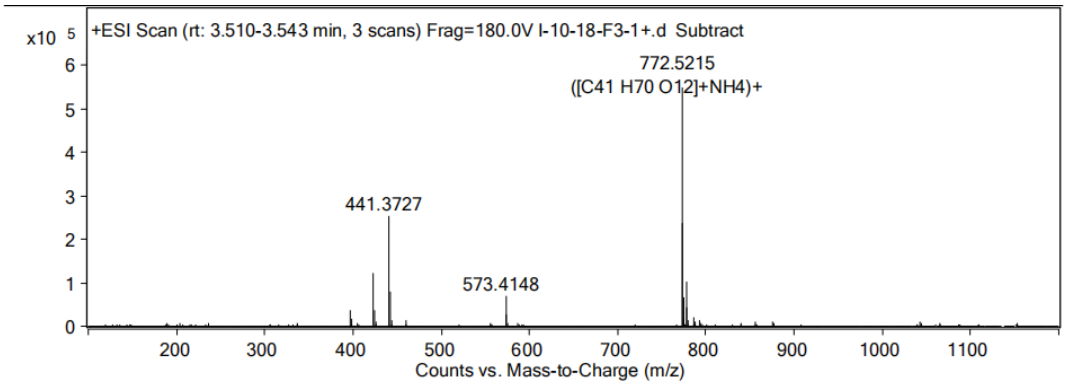


Figure S1: HRESIMS spectrum of compound **1**

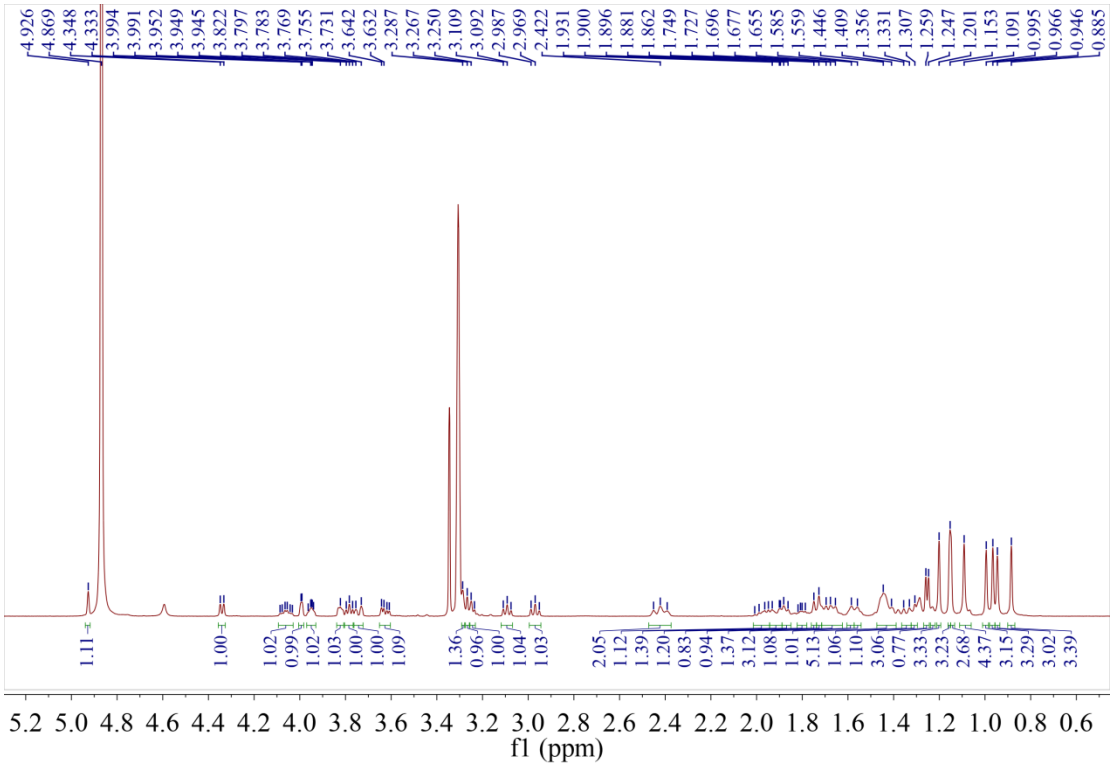


Figure S2: ^{1}H NMR spectrum of compound **1** (CD_3OD, 500MHz)

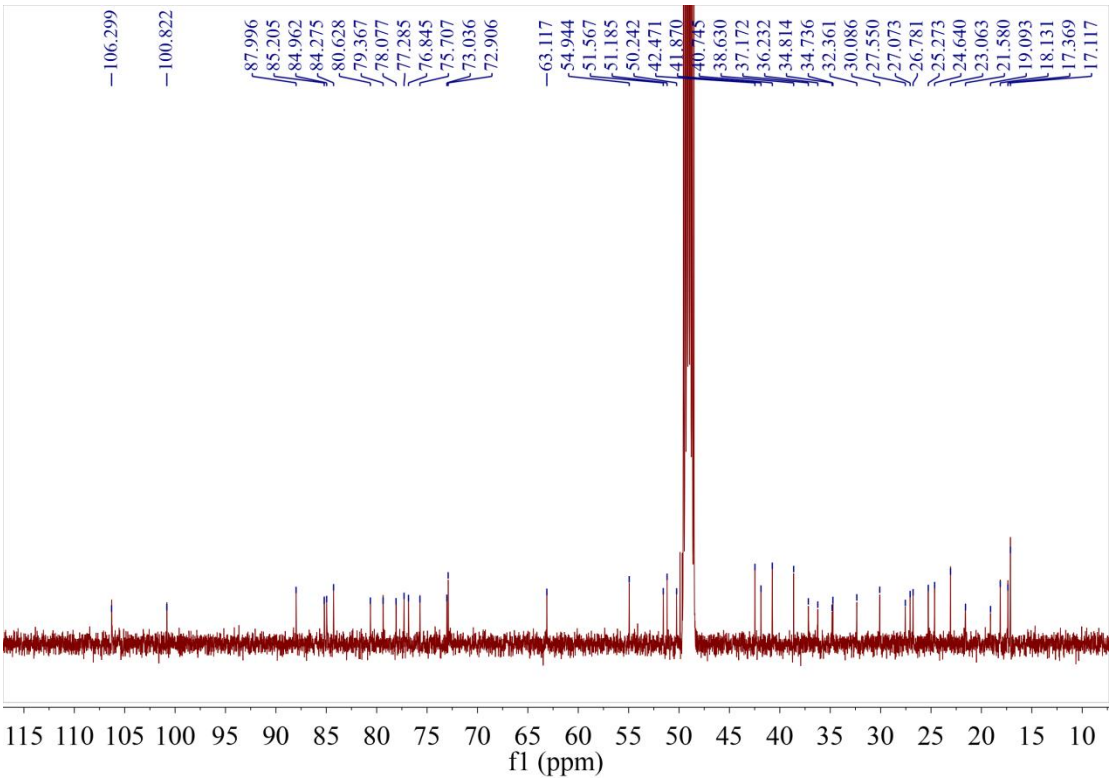


Figure S3: ^{13}C NMR spectrum of compound **1** (CD_3OD, 125MHz)

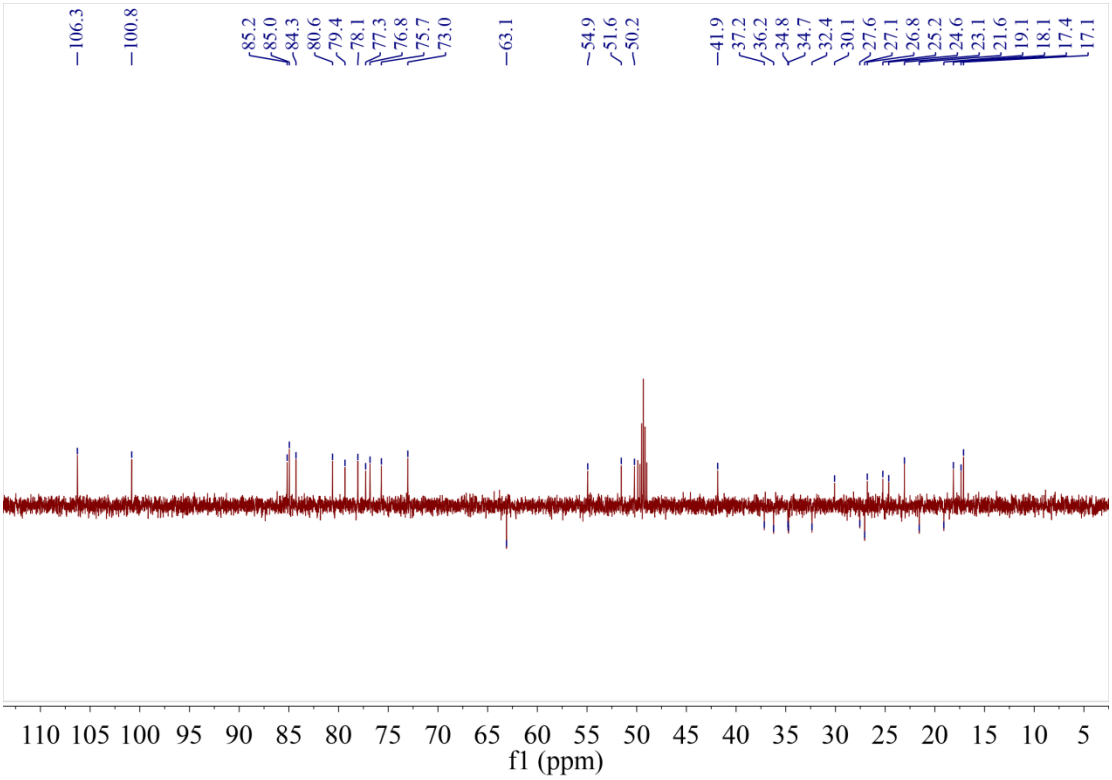


Figure S4: DEPT135 spectrum of compound **1** (CD_3OD, 125MHz)

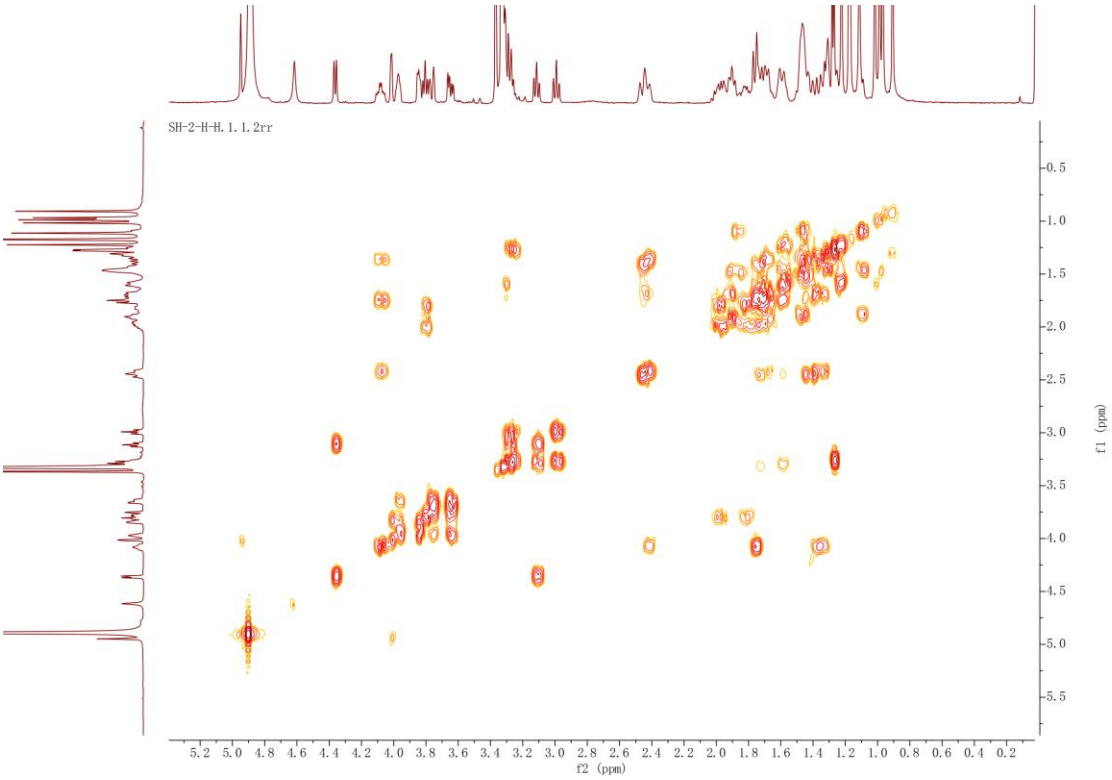


Figure S5: ^{1}H-^{1}H COSY spectrum of compound **1**

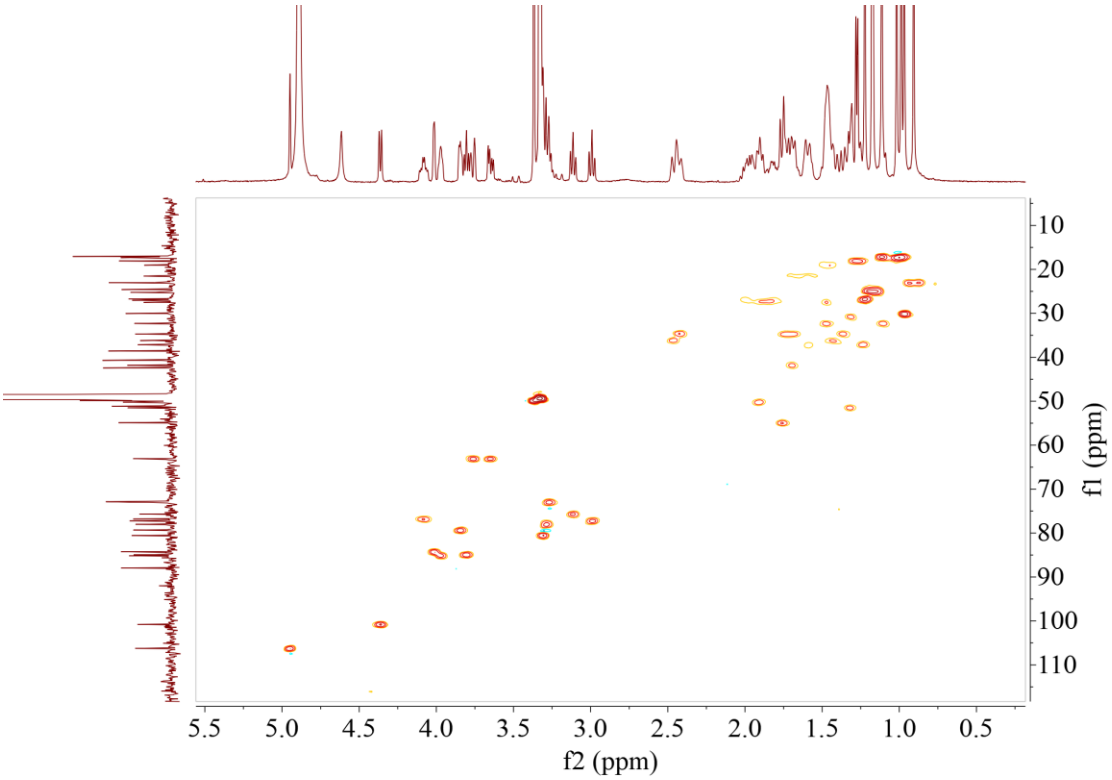


Figure S6: HSQC spectrum of compound **1**

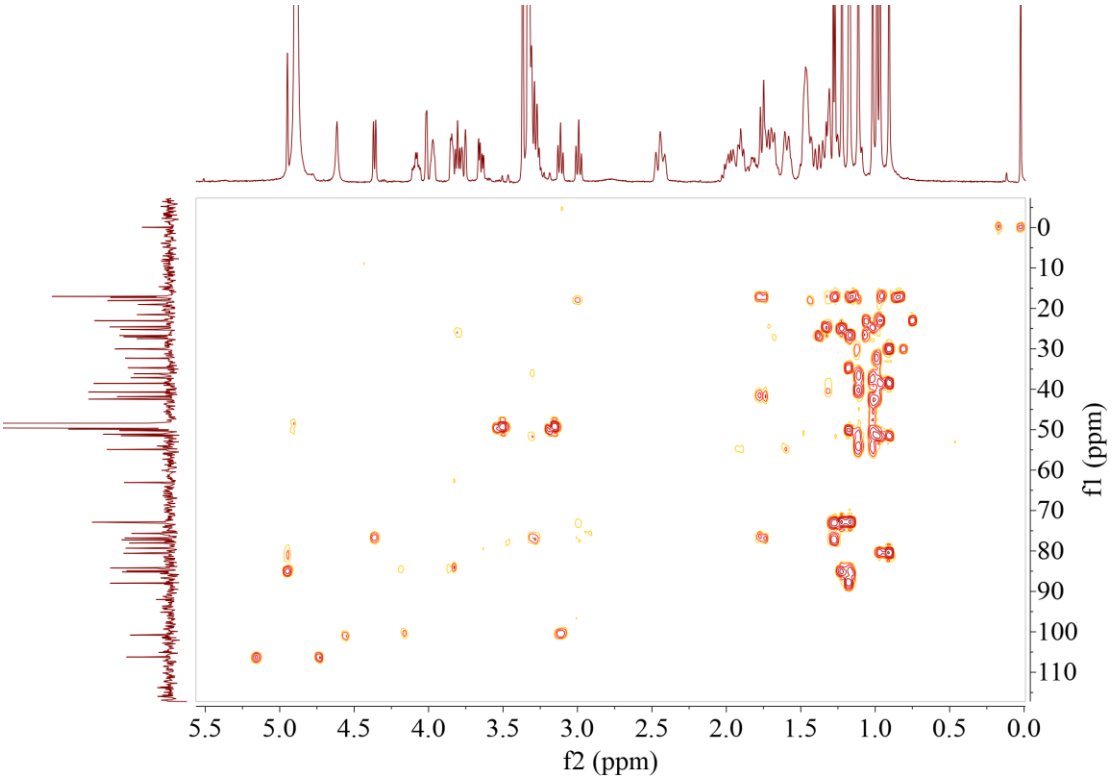


Figure S7: HMBC spectrum of compound **1**

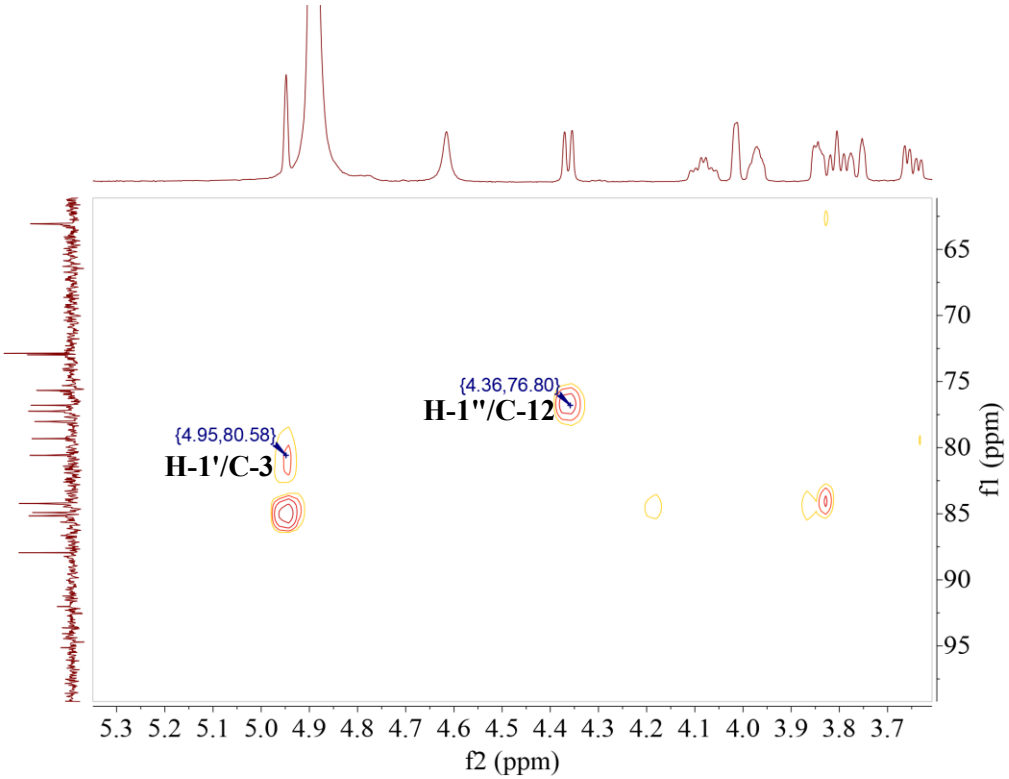


Figure S8: Local enlargement HMBC spectrum of compound **1**

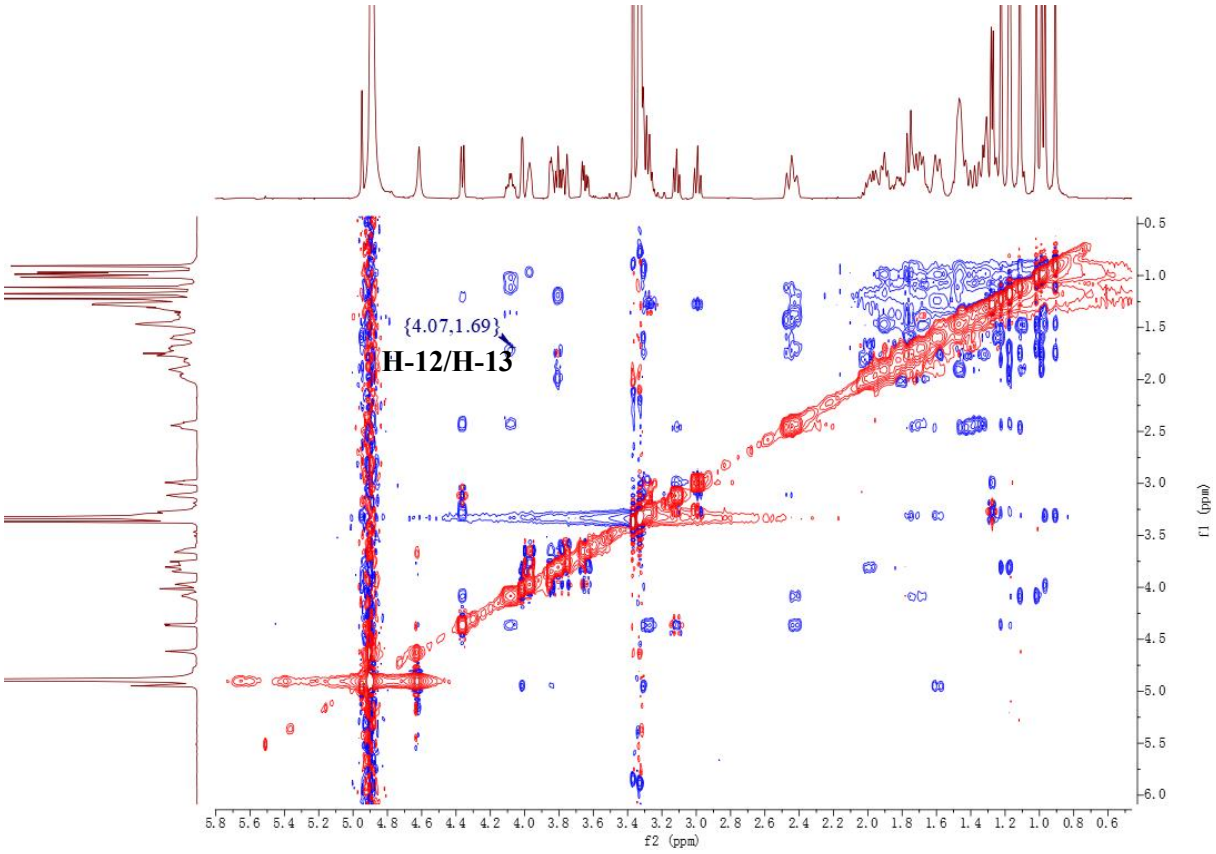


Figure S9: NOESY spectrum of compound **1**

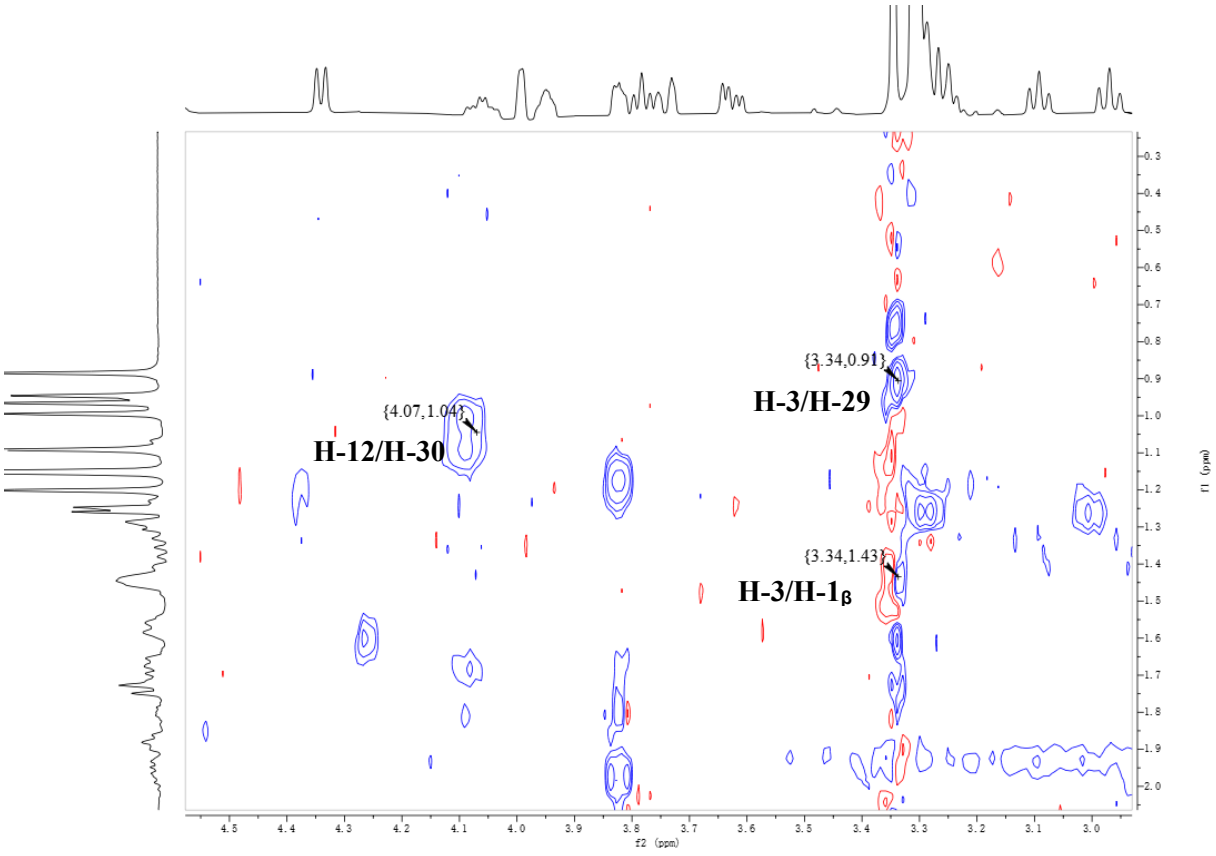
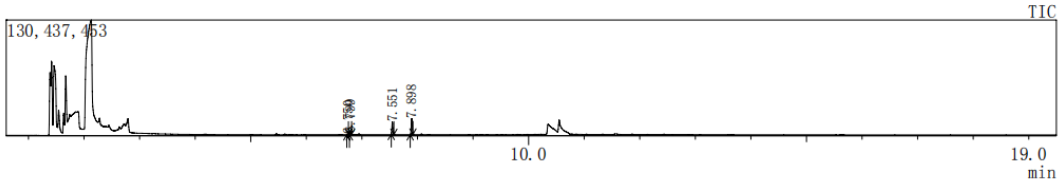


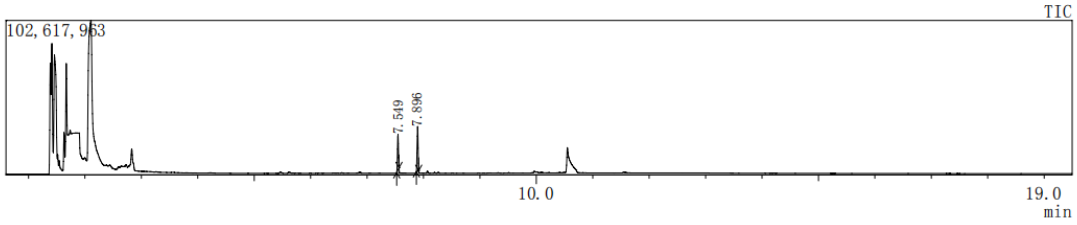
Figure S10: Local enlargement NOESY spectrum of compound **1**



峰表 TIC

峰号	保留时间	I. Time	F. Time	峰面积	峰面积%	峰高	峰高%	积/高度	标记	名称
1	6.750	6.730	6.760	371939	0.91	365186	1.08	1.02	MI	Arabinofuranose, 1,2,3,
2	6.799	6.775	6.815	2130616	5.20	1898393	5.59	1.12	MI	arabinofyranose, 1,2,3,
3	7.551	7.530	7.575	16649850	40.65	13927226	41.04	1.20	MI	Mannose, 6-deoxy-2,3,4,
4	7.898	7.870	7.920	21810492	53.24	17741898	52.29	1.23	MI	Mannose, 6-deoxy-2,3,4,
				40962897	100.00	33932703	100.00			

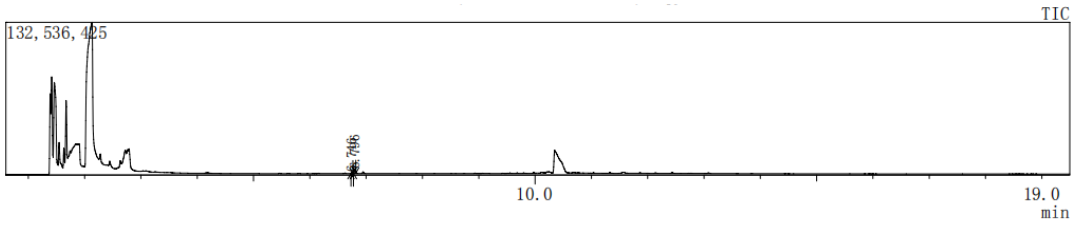
Figure S11: GC-MS spectrum of compound **1**



峰表 TIC

峰号	保留时间	I.Time	F.Time	峰面积	峰面积%	峰高	峰高%	积/高度	标记	名称
1	7.549	7.525	7.565	27670940	44.53	23417691	44.24	1.18	MI	Mannose, 6-deoxy-2,3,4,5-t
2	7.896	7.875	7.915	34465474	55.47	29520215	55.76	1.17	MI	Mannose, 6-deoxy-2,3,4,5-t
				62136414	100.00	52937906	100.00			

Figure S12: GC-MS chromatogram of D-quinovose (the monosaccharide standard)



峰表 TIC

峰号	保留时间	I.Time	F.Time	峰面积	峰面积%	峰高	峰高%	积/高度	标记	名称
1	6.746	6.730	6.765	918545	17.96	830064	18.66	1.11	MI	Arabinofuranose, 1,2,3,5-
2	6.796	6.775	6.815	4194792	82.04	3618884	81.34	1.16	MI	L-Arabinofuranose, 1,2,3,
				5113337	100.00	4448948	100.00			

Figure S13: GC-MS chromatogram of L-arabinofuranose (the monosaccharide standard)

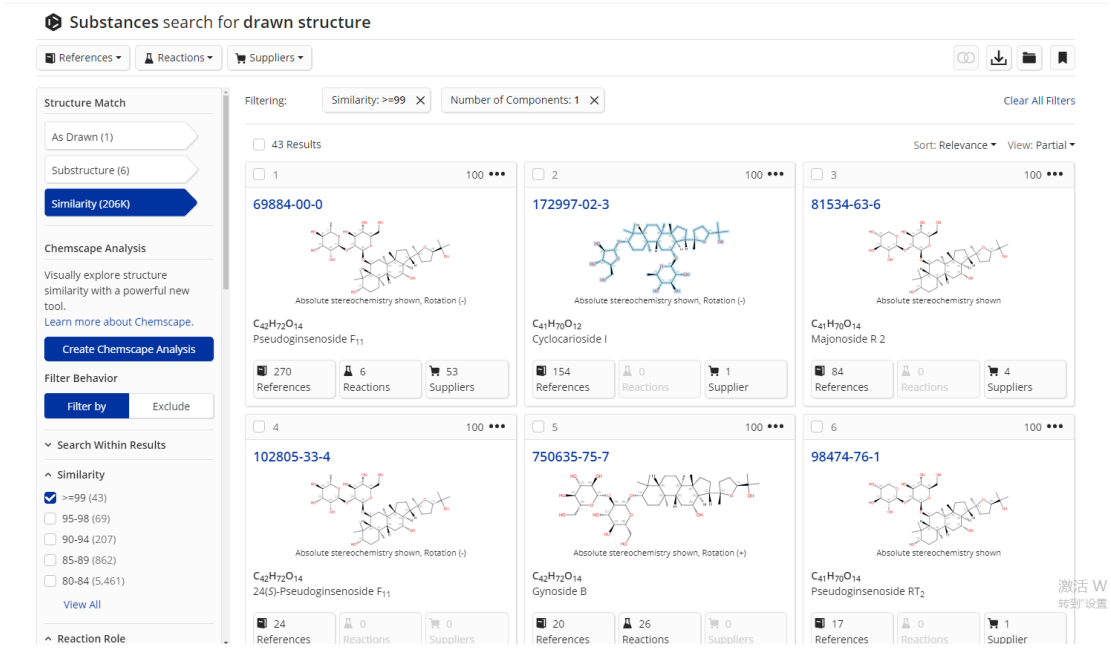
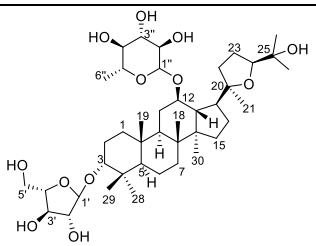
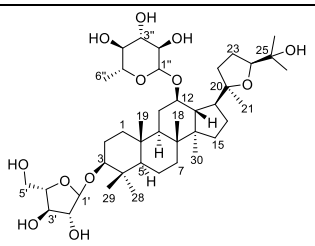


Figure S14: Scifinder similarity report for compound **1**

Table 1: ^{13}C-NMR data of **compound 1** (**cyclocarioside Z14**) and **cyclocarioside I**

Name	cyclocarioside Z14	cyclocarioside I
Structure		
Position	δ_C (CD_3OD, 125MHz)	δ_C (C_5D_5N, 125MHz)
1	36.2	35.7
2	27.0	26.7
3	80.6	79.3
4	38.6	38.0
5	51.5	51.0
6	19.1	18.4
7	37.1	36.4
8	42.4	41.6
9	54.9	54.1
10	40.7	10.0
11	34.7	23.5
12	76.8	76.8
13	41.8	41.2
14	51.5	50.2
15	32.3	31.6
16	27.0	21.3
17	50.2	49.3
18	17.3	17.0
19	17.1	16.7
20	88.0	86.4
21	24.6	24.5
22	34.7	34.2
23	21.5	26.3
24	84.9	84.2
25	73.0	71.2
26	25.2	26.1
27	26.7	27.6
28	30.0	30.0
29	23.0	23.0
30	17.3	16.8
1'	106.3	106.4
2'	84.2	83.9
3'	85.2	79.4
4'	79.3	85.8
5'	63.1	63.0
1"	100.8	101.5

2"	75.7	75.6
3"	78.0	78.4
4"	77.2	79.6
5"	72.9	72.8
6"	18.1	18.5