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### Materials and methods

#### General experimental procedures

Column chromatography (CC) was performed using silica gel (40-63  $\mu\text{m}$ ) or reversed phase C-18 (RP18, 150  $\mu\text{m}$ ) and Sephadex LH-20 as adsorbents. Thin-layer chromatography (TLC) was carried out on pre-coated silica gel 60 F<sub>254</sub>. For spot visualization, UV light and a vanillin- $\text{H}_2\text{SO}_4$  reagent were used.

NMR spectra were recorded using a Bruker Avance-500 spectrometer operating at frequencies of 500 MHz ( $^1\text{H}$ ) and 125 MHz ( $^{13}\text{C}$ ). The spectra were measured in  $\text{CD}_3\text{OD}$ .  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts ( $\delta$ ) were expressed in ppm with reference to solvent signal [ $\text{CDCl}_3$ :  $\delta\text{H}$  7.27 and  $\delta\text{C}$  77.3;  $\text{CD}_3\text{OD}$ :  $\delta\text{H}$  3.33 and  $\delta\text{C}$  49.0].

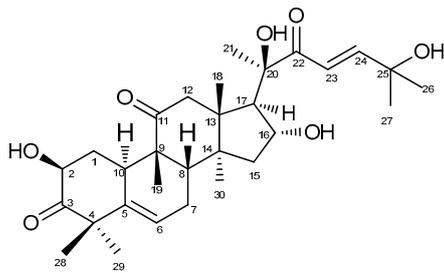
#### Plant material

The plant materials were collected in Ha Tinh province, Vietnam, in November 2021. *Elaeocarpus hainanensis* Oliv. species was botanically certified by Dr. Do Ngoc Dai, Department of Forestry, Nghe An University of Economics, and a voucher specimen (No. 04TN.EH21) was deposited in the Institute of Chemistry, VAST.

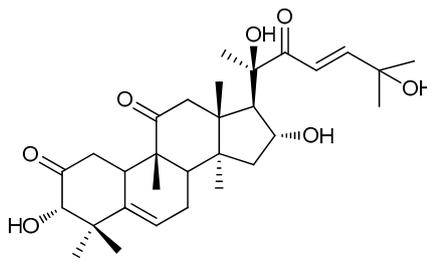
#### Extraction and isolation of cucurbitacins.

Dried twigs and leaves of *E. hainanensis* Oliv. (5.0 kg) were cut into pieces and extracted with methanol (MeOH) at room temperature, filtered, and the solvent was removed under

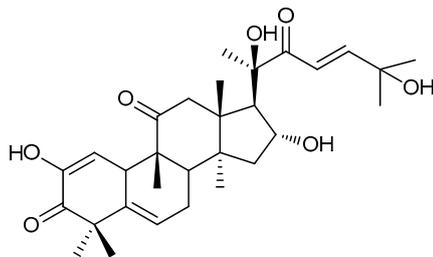
reduced pressure to give a crude MeOH extract (285g). This extract was suspended with water and sequentially partitioned with *n*-hexane and dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) to yield corresponding soluble fractions *n*-hexane (76 g), dichloromethane (ED, 60 g), and a residual water layer. Fraction ED was roughly chromatographed on a silica gel column, using a gradient solvent system of CH<sub>2</sub>Cl<sub>2</sub>-MeOH, 9/1→5/5, v/v) to obtain ten fractions ED1 – ED10. Fraction ED4 (10 g) was continuously separated on a silica gel column by using a gradient of CH<sub>2</sub>Cl<sub>2</sub>-acetone (1/9→5/5, v/v) to give 4 subfractions (ED4.1- ED4.4). Subfraction ED4.2 (1.15 g) was purified by a SiO<sub>2</sub> gel chromatography column using a gradient of CH<sub>2</sub>Cl<sub>2</sub>-acetone (5/5, v/v) to afford compound **1** (21 mg). Fraction ED5 (12 g) was re-chromatographed on a SiO<sub>2</sub> gel column using CH<sub>2</sub>Cl<sub>2</sub>-acetone (9/9→1/9, v/v) as an eluent to obtain six subfractions (ED5.1 – ED5.6). Subfraction ED5.2 (320 mg) was further purified by repeated column chromatography over Sephadex LH-20 to yield compound **2** (12 mg) and mixture compounds **1** and **3** (10 mg), respectively. The spectroscopic data for each isolate were compared to previously reported literature, including NMR (Table S1), HR ESI MS data: Cucurbitacin D (CucD, **1**), 3-*epi*-isocucurbitacin D (isocucD, **2**), and mixture of cucurbitacin D and cucurbitacin I (CucD + CucI, **1** + **3**). The ratio of compounds **1** and **3** in the mixture (**1**:**3** =1:1) is determined by the proton integral intensity (H-6).



**1**: Cucurbitacin D (CucD)



**2**: 3-*epi*-Isocucurbitacin D (Isocuc D)



**3**: Cucurbitacin I (CucI)

**Table S1.** <sup>13</sup>C (125 MHz) and <sup>1</sup>H (500 MHz) NMR data of compounds 1- 3

C	1, CDCl <sub>3</sub>		2, CD <sub>3</sub> OD		3, CD <sub>3</sub> OD	
	δ <sub>c</sub>	δ <sub>H</sub>	δ <sub>c</sub>	δ <sub>H</sub>	δ <sub>c</sub>	δ <sub>H</sub>
1	36.0	2.32 ddd (9.0, 6.0, 3.5) 1.21, d (7.0)	38.0	2.25 m, 2.21. m	116.7	5.99. d (2.5)
2	71.6	4.45 dd (6.0, 13.0)	213.0	-	146.8	-
3	213.0	-	81.2	4.10 s	216.1	-
4	50.3	-	42.5	-	47.4	-
5	140.5	-	141.1	-	140.0	-
6	120.3	5.78 dd (3.5,2.5)	122.9	5.92 t (5.2)	121.3	5.82 m
7	23.9	1.95 m 2.40 ddd (17.0, 5.5. 2.5)	24.8	2.05 m	24.6	1.94 m 2.40 m
8	42.4	1.97 m	44.2	2.01 m	43.3	2.07 m
9	48.3	-	49.5	-	48.8	-
10	33.8	2.78 br d (13.0)	34.1	3.13 m	35.9	2.86 m
11	212.2	-	215.7	-	212.0	-
12	48.7	3.31 d (14.5) 2.69 d (14.5)	49.9	2.59 m, 3.33 d (13.8)	48.6	3.42 d (14.5) 2.67d (14.5)
13	50.8	-	51.7	-	50.2	-
14	48.4	-	49.3	-	48.3	-
15	45.5	1.37 m, 1.87 dd (13.0, 8.5)	46.8	1.46m, 1.87 dd (12.0, 9.0)	46.6	1.41 m 1.89 m
16	71.5	4.34 t (7.3)	71.5	4.50 t (7.5)	71.7	4.50 m
17	57.3	2.56 d (7.3)	59.5	2.57 m	59.5	2.50 d (6.0)
18	20.0	0.98 s	20.7	0.92 s	20.3	0.95 s
19	20.1	1.08 s	19.4	1.09 s	20.7	1.01 s
20	78.1	-	79.9	-	79.9	-
21	24.0	1.39s	25.4	1.40 s	24.7	1.40 s
22	202.5	-	205.0	-	204.9	-
23	119.0	6.63 d (15.3)	121.3	6.84 d (15.5)	121.7	6.85d (15.2)
24	155.9	7.13 d (15.3)	155.3	6.98 d (15.5)	155.4	7.00 d (15.2)
25	71.2	-	71.7	-	71.7	-
26	29.5	1.35 s	29.2	1.34 s	29.3	1.39 s
27	29.3	1.35 s	29.2	1.35 s	29.1	1.33 s
28	28.9	1.30 s	19.0	1.41 s	28.3	1.34 s
29	21.3	1.34 s	24.7	0.96 s	21.6	1.29 s
30	19.3	1.34 s	27.6	1.24 s	18.9	1.34 s

QCCH428-CDC13-1H

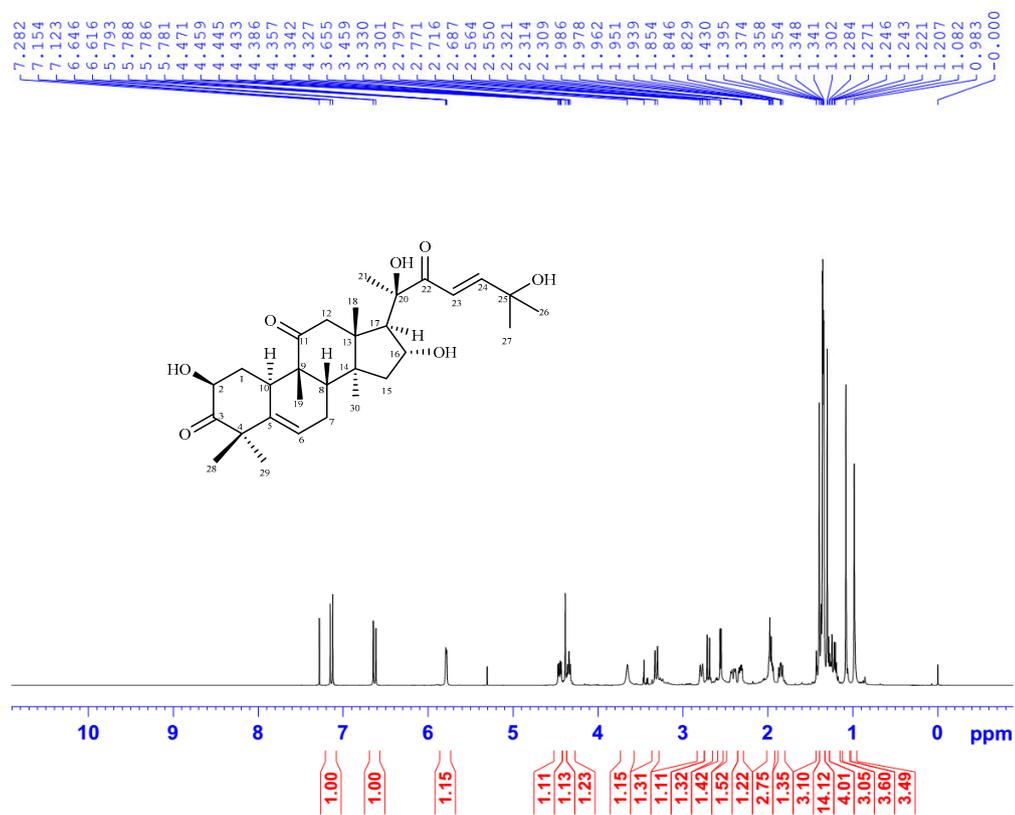
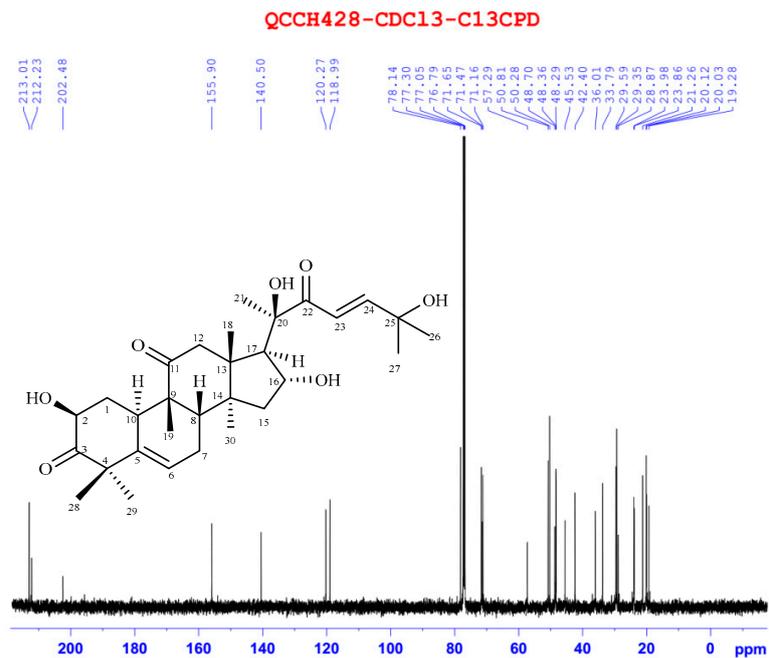


Figure S1. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) spectrum of CucD (1)



**Figure S2.**  $^{13}\text{C}$  NMR (125 MHz,  $\text{CDCl}_3$ ) spectrum of CucD (1)

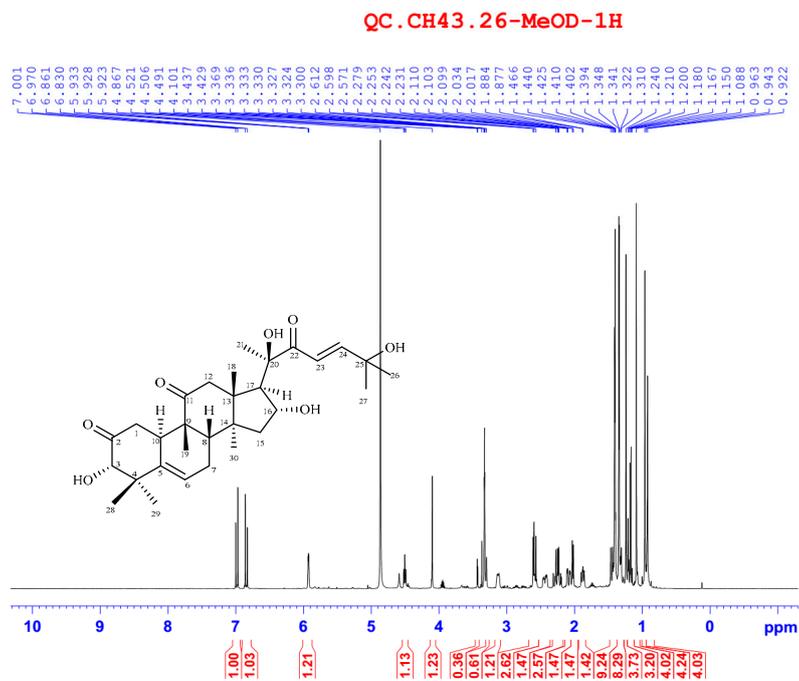


Figure S3.  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of IsocucD (2)

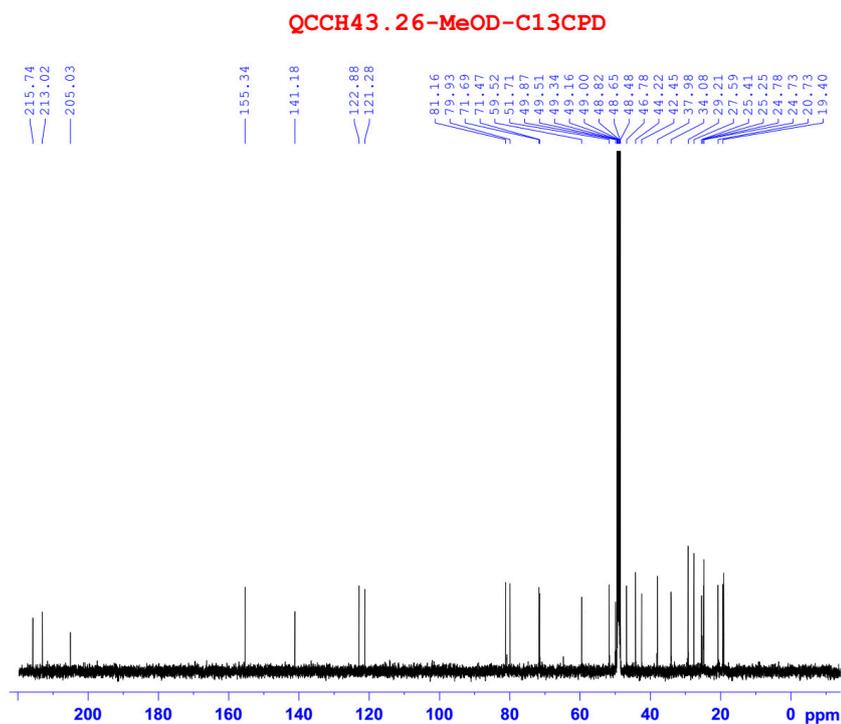


Figure S4.  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of IsocucD (2)

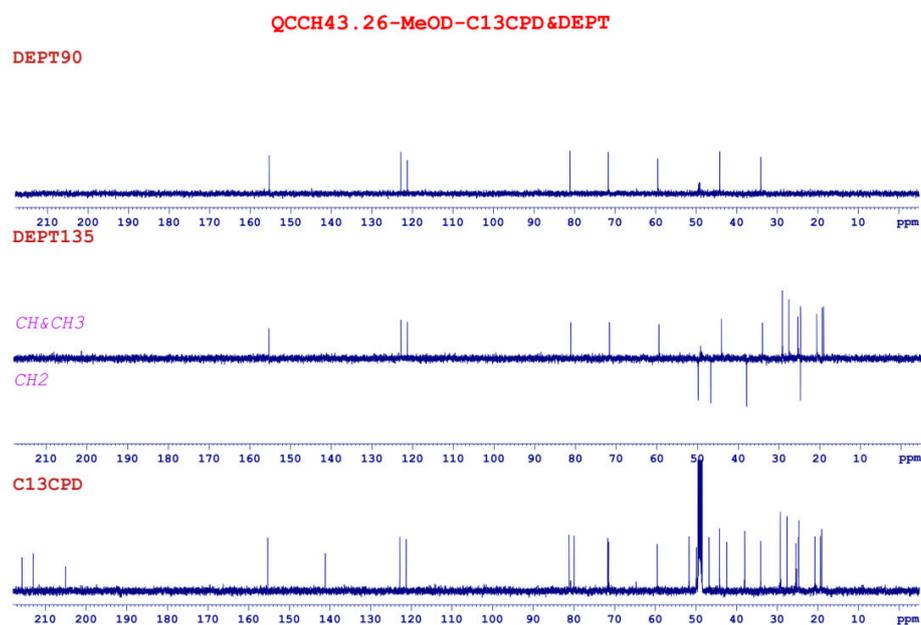
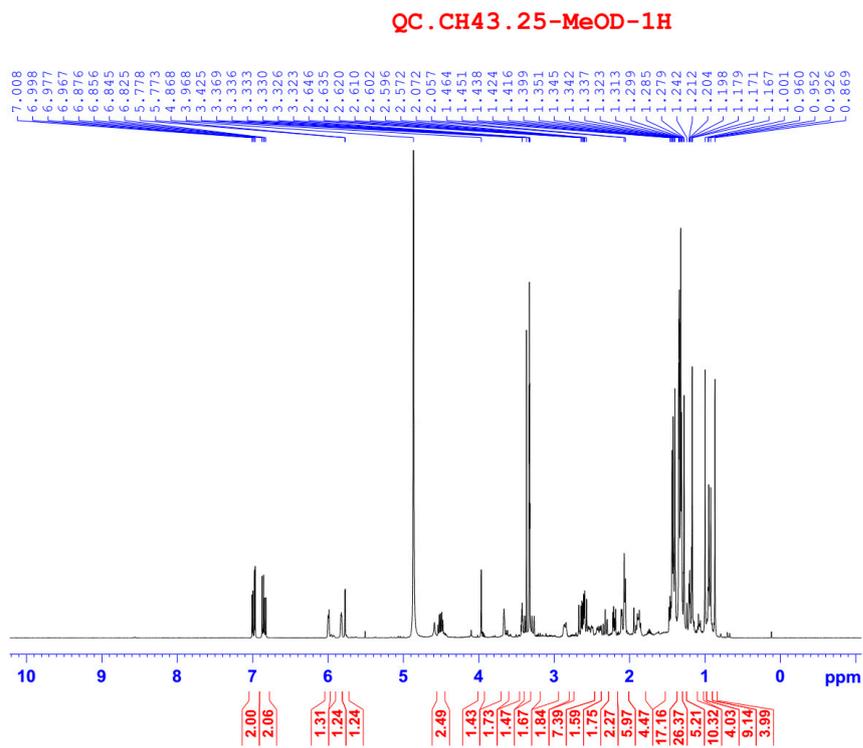
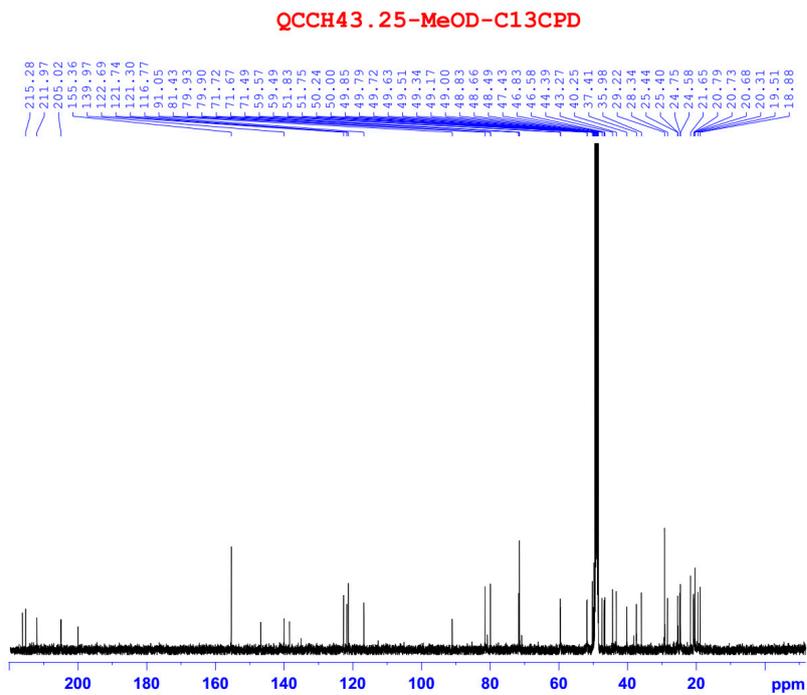


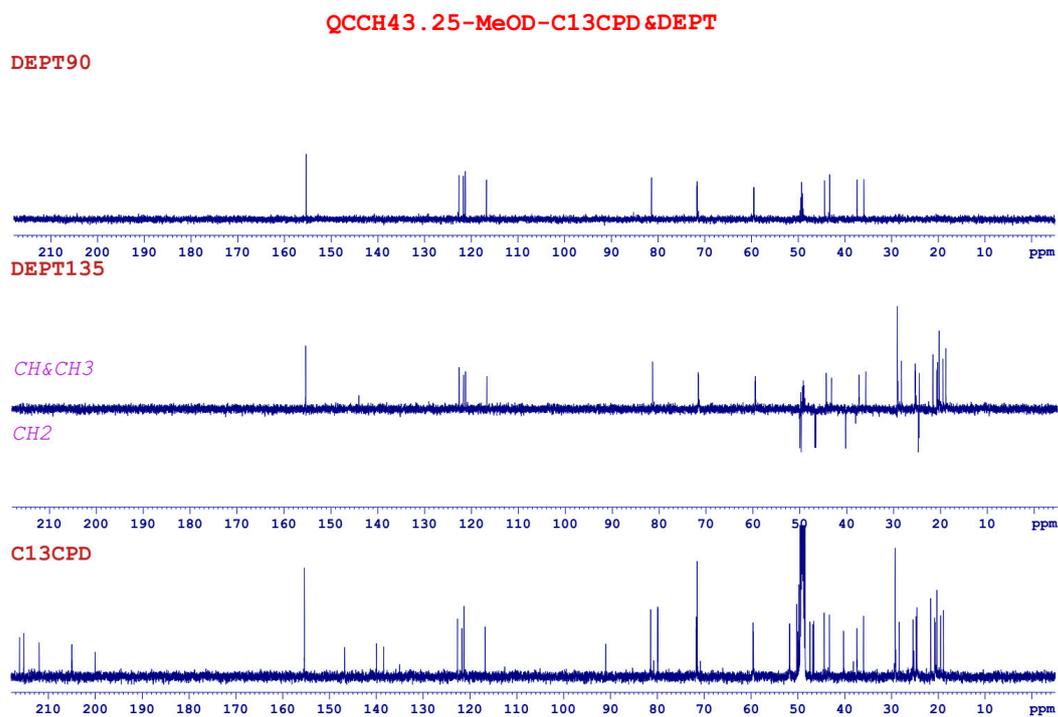
Figure S5. DEPT- $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of IsocucD (2)



**Figure S6.**  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of mixture CucD + CucI (3)



**Figure S7.**  $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of mixture CucD + CucI (3)



**Figure S8.** DEPT- $^{13}\text{C}$  NMR (125 MHz,  $\text{CD}_3\text{OD}$ ) spectrum of mixture CucD + CucI (3)