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Gneyulins A and B, Stilbene Trimers, and Noidesols A and B, Dihydroflavonol-*C*-Glucosides, from the Bark of *Gnetum gnemonoides*

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Gneyulins A (**1**) and B (**2**), two new stilbene trimers consisting of oxyresveratrol constituent units, and noidesols A (**3**) and B (**4**), two new dihydroflavonol-*C*-glucosides, were isolated from the bark of *Gnetum gnemonoides*. The structures and configurations of **1**−**4** were elucidated on the basis of 2D NMR correlations and X-ray analysis. Gneyulins A (**1**) and B (**2**) showed inhibition of Na^+-glucose transporters (SGLT-1 and SGLT-2).

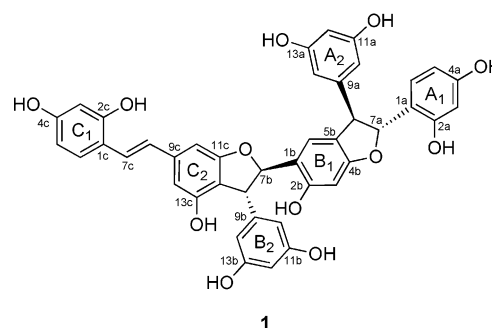
Gnetum species (Gnetaceae) produce a number of structurally diverse stilbenoids consisting of monomeric stilbenes such as resveratrol, oxyresveratrol, piceatannol, and isorhapontigenin.[1−5] Several monomeric and oligomeric stilbenes showed various biological activities such as hepatoprotective,[3] antioxidant,[6] antimicrobial,[7] and inhibition of tyrosinase,[4] lipase,[7] α-amylase,[7] and BACE1[8] activities.

In our search for structurally and biological interesting compounds from tropical plants found in Malaysia,[9] two new oxyresveratrol trimers, gneyulins A (**1**) and B (**2**), and two new dihydroflavonol-*C*-glucosides, noidesols A (**3**) and B (**4**), were isolated from the bark of *Gnetum gnemonoides*. We report here the isolation and structure elucidation of gneyulins A (**1**) and B (**2**) and noidesols A (**3**) and B (**4**) and the inhibitory activity of gneyulins A (**1**) and B (**2**) against Na^+-glucose cotransporter (SGLT).

The dried bark of *G. gnemonoides* was extracted with acetone/H_2O, and the extract was subjected to an LH-20 column ($CHCl_3$/MeOH) followed by π NAP HPLC (MeCN aq.) to afford gneyulins A (**1**, 0.0009% yield) and B (**2**, 0.002%), whereas the other fractions were subjected to a silica gel column ($CHCl_3$/MeOH), an ODS column (H_2O/MeOH), and then an ODS HPLC column (MeOH(aq)) to afford noidesols A (**3**, 0.03%) and B (**4**, 0.001%) together with resveratrol[10] and gnetin C.[11]

Gneyulin A (**1**), a brown, amorphous powder, had a molecular formula of $C_{42}H_{32}O_{12}$, by HRESITOFMS [m/z 751.1784 $(M + Na)^+$, Δ −0.7 mmu]. The ^1H NMR spectrum exhibited the presence of a set of two dihydrobenzofuran peaks of H-7a and H-7b (δ_H 5.73 and 5.80) and H-8a and H-8b (δ_H 4.39 and 4.42), and *trans*-coupled (16.4 Hz) olefinic protons, H-7c and H-8c (δ_H 7.36 and 6.96). The presence of a set of two 1,2,4-trisubstituted benzene moieties (ring A_1: δ_H 6.33, 6.42, and 7.07; ring C_1: δ_H 6.41, 6.46, and 7.42) and a set of two symmetrical 1,3,5-trisubstituted benzene units (ring A_2: δ_H 6.19 and 6.23; ring B_2: δ_H 6.31 and 6.23) was also indicated. All protonated carbons were assigned by the HSQC spectrum.

The presence of two dihydrobenzofuran rings (B_1 and C_2) and their connectivities between C-7b and C-1b and between C-8c and C-9c were elucidated by HMBC correlations as shown in Figure 1. HMBC correlations of H-10a to C-8a and H-6a to C-7a indicated the connection between rings A_1 and A_2, and a dihydrofuran ring.



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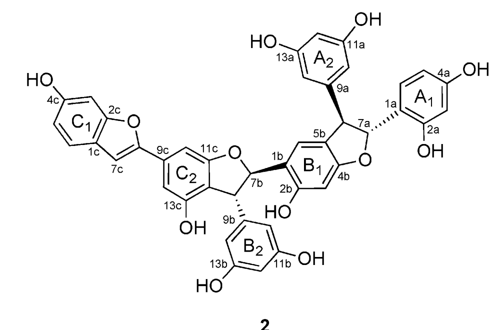

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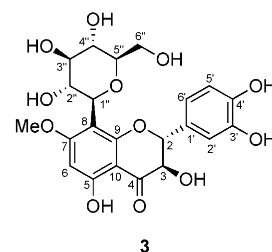

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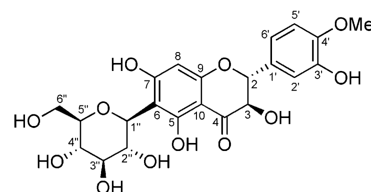

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HMBC correlations of H-10b to C-8b and H-6c to C-7c established the connections between ring B_2 and C-8b and between ring C_1

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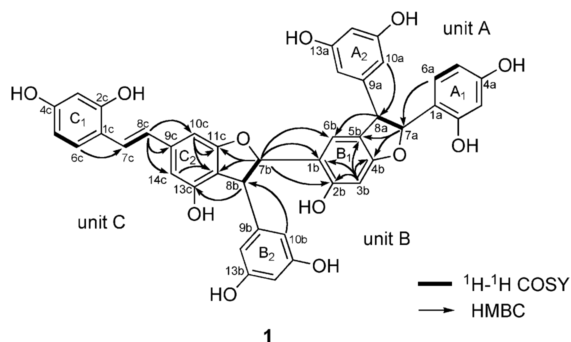

Figure 1. Selected 2D NMR correlations for gneyulin A (**1**).

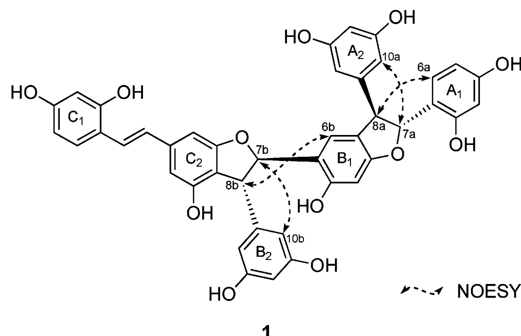

Figure 2. Selected NOESY correlations for gneyulin A (**1**).

and C-7c, respectively. Since all the unsaturations of gneyulin A were accounted for, **1** was inferred to possess 10 hydroxy groups at C-2a, C-4a, C-11a, C-13a, C-2b, C-11b, C-13b, C-2c, C-4c, and C-13c. Thus, the gross structure of **1** was elucidated as an oxyresveratrol trimer, as shown in Figure 1. The phase-sensitive NOESY spectrum showed cross-peaks as shown in the 3D drawing of **1** (Figure 2). NOESY correlations for H-7a/H-10a, H-8a/H-6a, H-7b/H-10b, and H-8b/H-6b revealed the *trans*-configuration of the two dihydrofuran rings.

The molecular formula of gneyulin B (**2**) was smaller than that of gneyulin A (**1**) by two mass units. ^{1}H and ^{13}C NMR data (Table 1) of **2** were analogous to those of **1** with an sp^2 methine (δ_C 102.2, δ_H 6.95) and an oxygen-bearing sp^2 quaternary carbon (δ_C 156.7) replacing the two *trans*-coupled methines (H-7c and H-8c) of **1**. The gross structure of **2** was elucidated by 2D NMR (^{1}H−^{1}H COSY, HSQC, and HMBC) data. HMBC correlations of H-10c, H-14c, and H-7c to C-8c and H-7c to C-2c indicated the presence of a C-1 substituted benzofuran ring in unit C. Furthermore, ^{1}H and ^{13}C NMR data of unit C in **2** were similar to moracin M (6,3′,5′-trihydroxy-2-phenylbenzofuran).[12,13] Thus, the structure of gneyulin B (**2**) was assigned as the 1-benzofuran analogue of **1**. Gneyulins A (**1**) and B (**2**) are the first homooxyresveratrol trimers.

Noidesol A (**3**) was obtained as colorless platelets and showed a positive reaction with the Gibbs reagent. It had a molecular formula of $C_{22}H_{24}O_{12}$, by HRESITOFMS [*m/z* 503.1167 (M + Na)$^+$, Δ +0.2 mmu]. IR absorptions implied the presence of a conjugated carbonyl (1636 cm^{-1}) and hydroxy (3410 cm^{-1}) groups. Multiple pairs of signals were observed in the ^{1}H and ^{13}C NMR spectra of **3** in DMSO-d_6 in a ratio of ca. 3:1, suggesting that **3** existed as a mixture of two rotamers (Table 2). This phenomenon is similar to spinosin,[14] showing a rotational barrier of the flavone−6-*C*-glycoside bond. ^{1}H and ^{13}C NMR signals of the two conformers in **3** were assigned by analyses of the 2D NMR (^{1}H−^{1}H-COSY, TOCSY, and HSQC) spectra. HMBC correlations of methoxy protons to C-7 and H-1″ to C-7 and C-8 indicated the presence of a methoxy group at C-7 and a *C*-glucose at C-8, as shown in Figure 3. Thus, the gross structure of **3** was assigned as a 8-*C*-glucosyl-7-methoxydihydroflavonol. The 2,3-configuration was elucidated as *trans* by a large coupling constant (11.3 Hz) between H-2 and H-3.

Table 1. ^{1}H (J, Hz) and ^{13}C NMR Data of Gneyulins A (**1**) and B (**2**) at 300 K

	1[a]		**2**[b]	
	δ_H	δ_C	δ_H	δ_C
1a		118.7		119.7
2a		155.9		157.3
3a	6.42, d (1.9)	102.7	6.27, d (2.3)	103.6
4a		158.2		159.4
5a	6.33, dd (8.3, 1.9)	106.6	6.23, dd (8.4, 2.3)	107.5
6a	7.07, d (8.3)	128.0	7.00, d (8.4)	129.2
7a	5.73, d (6.0)	89.0	5.64, d (6.5)	90.7
8a	4.39, d (6.0)	55.0	4.35, d (6.5)	56.2
9a		146.5		147.5
10a (14a)	6.19, d (2.1)	106.4	6.10, d (2.3)	107.6
11a (13a)		158.6		159.4
12a	6.23, dd (2.1, 2.1)	101.2	6.24, dd (2.3, 2.3)	102.0
1b		121.3		122.3
2b		154.7		156.2
3b	6.53, s	96.9	6.40, s	97.8
4b		160.6		161.7
5b		121.2		122.1
6b	6.93, s	122.2	6.83, s	123.4
7b	5.80, d (2.8)	88.3	5.72, d (3.4)	90.2
8b	4.42, d (2.8)	53.8	4.40, d (3.4)	55.0
9b		146.2		147.1
10b (14b)	6.31, d (2.1)	106.3	6.24, d (2.2)	107.4
11b (13b)		158.3		159.2
12b	6.23, dd (2.1, 2.1)	100.9	6.14, dd (2.2, 2.2)	101.8
1c		116.5		123.1
2c		156.1		156.4
3c	6.46, d (2.0)	102.8	6.90, m	98.5
4c		158.2		157.2
5c	6.41, dd (8.4, 2.0)	107.6	6.72, dd (8.4, 2.2)	113.2
6c	7.42, d (8.4)	127.6	7.34, d (8.4)	122.0
7c	7.36, d (16.4)	123.5	6.95, brs	102.2
8c	6.96, d (16.4)	125.6		156.7
9c		141.0		133.8
10c	6.65, s	98.4	6.89, m	98.2
11c		162.4		163.5
12c		114.5		117.0
13c		154.8		156.1
14c	6.55, s	106.7	6.83, d (1.3)	105.8

[a] In acetone-d_6. [b] In CD_3OD.

NOESY correlations between H-1″ and 7-OMe (**3a**: major conformer) and between H-2″ and 7-OMe (**3b**: minor conformer) were observed for each confomational isomer, which was elucidated to be *P*- and *M*-helicity, respectively (Figure 4).

The structure of **3** was assigned by X-ray analysis of a crystal of **3** obtained from MeOH/H_2O (Figure 5). The absolute configurations at C-2 and C-3 were deduced as (2*R*, 3*R*), respectively, through the Flack parameter[15] $x = -0.1(2)$. The X-ray crystallographic structure as shown in Figure 5 was corresponding to the major conformer in DMSO-d_6 analyzed by NOESY correlations (Figure 4).

HRESITOFMS data [*m/z* 503.1200 (M + Na)$^+$, Δ +1.0 mmu] of noidesol B (**4**) established the molecular formula to be $C_{22}H_{24}O_{12}$, which was the same as noidesol A (**3**). Noidesol B (**4**) gave well-resolved sharp signals indicative of a single conformer. The complete assignment of the NMR signals is shown in Table 2. Analysis of 2D NMR (^{1}H−^{1}H COSY and HSQC) spectra and HMBC correlations of methoxy protons to C-4′ and H-1″ to C-5, C-6, and C-7 indicated the presence of a methoxy group at C-4′ and *C*-glucose at C-6 as shown in Figure 6. Thus, the gross structure of **4** was assigned as 6-*C*-glucosyl-4′-methoxydihydroflavonol. The 2,3-*trans* configuration was also indicated by the large coupling constant between H-2 and H-3 (11.2 Hz). The absolute configuration of C-2 and C-3 was determined to be (2*R*, 3*R*) by the CD spectrum with a positive Cotton effect at 331 nm (θ 6900) and a negative

Table 2. ^{1}H (J, Hz) and ^{13}C NMR Data of Major (**3a**) and Minor (**3b**) Conformers of Noidesol A in DMSO-d_6 and Noidesol B (**4**) in CD_3OD at 300 K

	3a		**3b**		**4**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
2	5.02, d (11.3)	82.1	5.02, d (11.3)	82.9	5.04, d (11.2)	84.3
3	4.24, dd (11.3, 6.6)	72.7	4.38, dd (11.3, 6.6)	71.9	4.42, d (11.2)	73.7
4		198.9		198.6		198.6
5		163.1		163.3		163.0
6	6.21, s	92.4	6.21, s	93.4		105.8
7		166.2		167.6		167.8
8		106.8		106.6	6.00, s	97.5
9		161.0		160.2		164.6
10		101.6		101.0		101.9
1′		128.7		128.0		131.3
2′	6.94, d (2.0)	115.1	6.87, d (2.0)	115.3	7.11, s	115.6
3′		144.8		145.1		147.4
4′		145.2		145.8		149.4
5′	6.70, d (8.2)	115.2	6.72, d (8.1)	115.2	6.95, d (7.6)	112.4
6′	6.84, dd (8.2, 1.9)	118.2	6.77, dd (8.2, 1.9)	119.2	7.04, d (7.6)	120.4
1″	4.45, d (9.7)	72.8	4.42, d (9.7)	73.1	4.73, d (9.7)	75.2
2″	3.77, m	70.0	3.89, ddd (9.7, 8.8, 5.0)	70.5	4.02, dd (9.3, 9.0)	72.5
3″	3.08, m	78.7	3.08, m	79.1	3.36, dd (9.2, 9.0)	79.9
4″	2.82, ddd (9.2, 9.2, 5.2)	70.7	2.99, m	71.0	3.31, m	71.9
5″	3.10, m	81.6	2.98, m	81.8	3.32, m	82.2
6″	3.41, m	62.2	3.31, m	61.9	3.71, brd (9.1)	63.1
	3.73, ddd (11.6, 5.8, 1.7)		3.63, dd (11.6, 5.7)		3.85, m	
7-OCH_3	3.82, s	56.6	3.78, s	56.4		
4′-OCH_3					3.87, s	56.5
3-OH	5.88, d (6.6)		5.88, d (6.6)			
5-OH	12.09, s		12.16, s			
3′-OH	8.80, s		8.96, s			
4′-OH	8.94, s		8.94, s			
2″-OH	4.57, d (5.4)		4.58, d (5.0)			
3″-OH	4.78, d (3.7)		4.79, d (4.4)			
4″-OH	4.79, d (5.3)		4.76, d (5.1)			
6″-OH	4.49, dd (5.8, 5.7)		4.37, m			

Cotton effect at 295 nm (θ −29 000), similar to those of noidesol A [**3**: 333 (θ 8700), 296 (θ −35 000)].[16] Although a variety of *C*-glycosyl flavonoids have been isolated from various plants,[17−20] dihydroflavonol *C*-glucosides with a glucose moiety at C-6 or C-8 are rare.

SGLT is a membrane protein that plays an important role in the reabsorption of glucose in the kidneys. SGLT is known to have three isoforms (SGLT1, SGLT2, and SGLT3).[21−23] SGLT1 is expressed primarily in the brush border membrane of mature enterocytes in the small intestine, where it absorbs dietary glucose and galactose from the gut lumen.[24] SGLT2 is only expressed in the renal cortex, where it is assumed to be present in the brush border membrane of the S1 and S2 segments of the proximal tubule and to be responsible for the reabsorption of glucose from the glomerular filtrate.[24] SGLT inhibitors have therapeutic potential for type 2 diabetes.[25] The oxyresveratrol trimers gneyulins A (**1**) and B (**2**) showed moderate inhibitory activity against SGLT1 and SGLT2 (**1**: IC_{50} 27 and 25 μM, respectively; **2**: 37 and 18 μM, respectively), whereas noidesols A and B did not.[26]

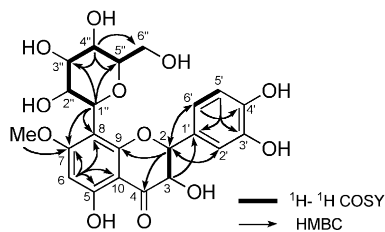

Figure 3. Selected 2D NMR correlations for noidesol A (**3**).

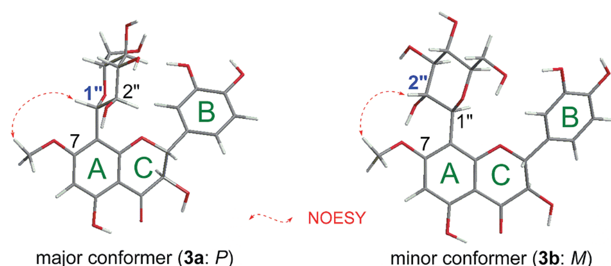

Figure 4. Selected NOESY correlations and two conformers (**3a** and **3b**) proposed for noidesol A (**3**).

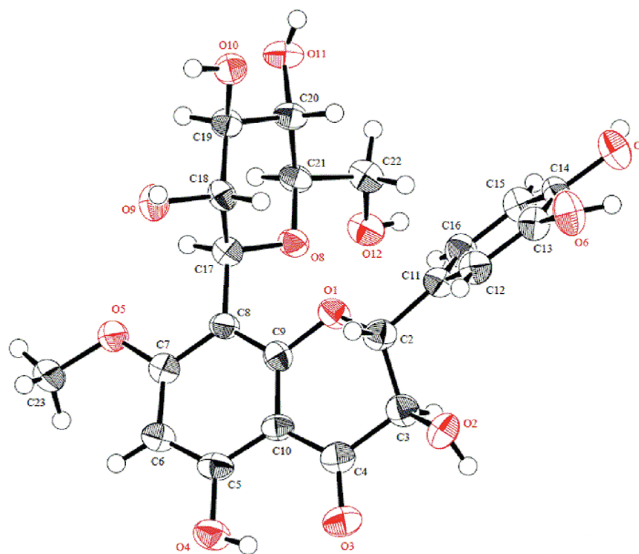

Figure 5. Molecular structure of noidesol A (**3**) obtained by X-ray analysis [Flack parameter: x = −0.1(2)].

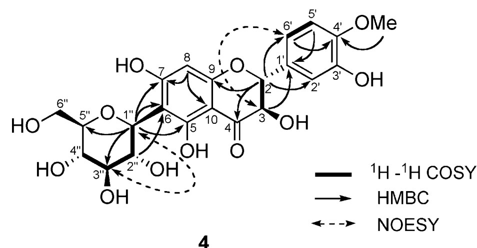

Figure 6. Selected 2D NMR correlations for noidesol B (**4**).

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 polarimeter. UV spectra were recorded on a Shimadzu UVmini-1240 spectrophotometer and IR spectra on a JASCO FT/IR-4100 spectrophotometer. CD spectra were recorded on a JASCO J-820 polarimeter. Mass spectra were obtained using a Waters ZQ-2000 and a LTQ Orbitrap XL (Thermo Scientific) spectrometer. ^{1}H and 2D NMR spectra were recorded on JEOL ECA 600 and Bruker AV 400 spectrometers, and chemical shifts were referenced to the residual solvent peaks (δ_H 3.31 and δ_C 49.0 for methanol-d_4, δ_H 2.06 and δ_C 206.1 for acetone-d_6, and δ_H 2.49 and δ_C 39.7 for DMSO-d_6). Standard pulse sequences were employed for the 2D NMR experiments. HPLC was performed on a CAPCELL PAK C_{18} MG-II, 5 μm (Φ 10 $\times$ 250 mm) and COSMOSIL π NAP, 5 μm (Φ 10 $\times$ 250 mm).

Material. Bark of *G. gnemonoides* was collected in Mersing, Malaysia, in 2007. The botanical identification was made by Mr. Teo Leong Eng, Faculty of Science, University of Malaya. A voucher specimen (Herbarium No. KL 5468) is deposited at the Herbarium of the Department of Chemistry, University of Malaya, Kuala Lumpur, Malaysia.

Extraction and Isolation. The dried bark of *G. gnemonoides* (187 g) was extracted with acetone/H_2O (7:3, 3 $\times$ 1 L) at rt, and the extract subjected to an LH-20 column in $CHCl_3$/MeOH (1:1) to yield 14 fractions. The 13th fraction was separated by π NAP HPLC (45% MeCN aq., 2.0 mL/min, 290 nm) to afford gneyulins A (**1**, 1.7 mg, 0.0009%, t_R = 20.5 min) and B (**2**, 3.5 mg, 0.002%, t_R = 37.2 min). The fifth fraction was applied to a silica gel column in $CHCl_3$/MeOH (10:1 → 0:1). The $CHCl_3$/MeOH (8:2) eluted fraction was chromatographed over an ODS column in H_2O/MeOH (10:1 → 0:1), and the H_2O/MeOH (10:1) eluted fraction was separated by an ODS HPLC column (28% MeCN aq., 2.0 mL/min, 254 nm) to afford noidesol A (**3**, 50.7 mg, 0.03%, t_R = 13.1 min), whereas the $CHCl_3$/MeOH (7:3) eluted fraction was chromatographed over an ODS column in H_2O/MeOH (7:3 → 0:1) and the H_2O/MeOH (7:3) eluted fraction was separated by an ODS HPLC column (20% MeCN aq., 2.0 mL/min, 254 nm) to afford noidesol B (**4**, 2.5 mg, 0.001%, t_R = 61.6 min). The eighth fraction was applied to a silica gel column in $CHCl_3$/MeOH (1:0 → 0:1) to afford resveratrol (31.5 mg, 0.02%)[9] and gnetin C (34.0 mg, 0.02%).[10]

Gneyulin A (1): brown, amorphous powder; $[\alpha]_D^{27}$ +47 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.93), 288 (4.17), and 332 (4.18) nm; CD (MeOH) λ_{max} 202 (θ 90 000), 214 (−170 000), 242 (49 000), 288 (−6500), 321 (16 000), 340 (16 000) nm; IR (KBr) ν_{max} 3333, 1614, 1158, and 693 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 751 (M + Na)$^+$; HRESITOFMS *m/z* 751.1784 (M + Na; calcd for $C_{42}H_{32}O_{12}$Na, 751.1791).

Gneyulin B (2): brown, amorphous powder; $[\alpha]_D^{27}$ −316 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 205 (4.89), 287 (4.17), 321 (4.33), and 332 (4.19) nm; CD (MeOH) λ_{max} 203 (θ 91 000), 215 (−150 000), 245 (49 000), 298 (−13 000), 321 (16 000), 334 (18 000) nm; IR (KBr) ν_{max} 3397, 1614, 1149, and 691 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 1); ESIMS *m/z* 725 (M − H)$^-$; HRESITOFMS *m/z* 727.1794 (M + H; calcd for $C_{42}H_{31}O_{12}$, 727.1816).

Noidesol A (3): colorless platelets, mp 178 °C (from MeOH/H_2O); $[\alpha]_D^{26}$ −31 (*c* 0.5, MeOH); UV (MeOH) λ_{max} (log ε) 204 (4.67), 291 (4.22), and 328 (3.57) nm; CD (MeOH) λ_{ext} 215 (θ −22 000), 230 (9700), 296 (−35 000), 333 (8700) nm; IR (KBr) ν_{max} 3410, 2871, 1636, 1205, and 1115 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); ESIMS *m/z* 503 (M + Na)$^+$; HRESITOFMS *m/z* 503.1167 (M + Na; calcd for $C_{22}H_{24}O_{12}$Na, 503.1165).

Noidesol B (4): yellow, amorphous powder, $[\alpha]_D^{29}$ +8.6 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 202 (4.62), 291 (4.05), and 328 (3.79) nm; CD (MeOH) λ_{max} 213 (θ −21 000), 225 (11 000), 295 (−29 000), 331 (6900) nm; IR (KBr) ν_{max} 3387, 2963, 1643, 1261, 1030, and 800 cm^{-1}; ^{1}H and ^{13}C NMR data (Table 2); ESIMS *m/z* 503 (M + Na)$^+$; HRESITOFMS *m/z* 503.1200 (M + Na; calcd for $C_{22}H_{24}O_{12}$Na, 503.1190).

X-ray Analysis of Noidesol A (3). Noidesol A (**3**) was crystallized from MeOH/H_2O to give colorless platelets. Crystal data: $C_{23}H_{28}O_{13}$, space group $P2_12_12_1$ (#19), a = 9.31894(17) Å, b = 11.1351(2) Å, c = 22.5910(7) Å, V = 2344.21(9) Å^3, Z = 4, D_{calc} = 1.452 g/cm^3, Cu Kα radiation (λ = 1.54187 Å), T = −180(1) °C. The structure was solved by direct methods and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement on F^2 was based on 4210 observed reflections and converged with unweighted and weighted agreement factors of R1 = 0.0471 [$I > 2.00\sigma(I)$] and wR2 = 0.1000. The absolute configuration was determined based on a Flack parameter of −0.1(2),[14] refined using 1788 Friedel pairs. Complete crystallographic data of **3** have been deposited in the Cambridge Crystallographic Data Centre (CCDC 747369).[27]

Uptake of Methyl-α-D-glucopyranoside in Cultured Cells Expressing SGLT1 or SGLT2.[26] COS-1 cells were cultured at 37 °C in Dulbecco's modified Eagle's/Ham's F-12 medium (1:1) supplemented with 10% fetal calf serum. For the uptake assay, the cells were plated at 1 $\times$ 10^5 cells/24-well plate (Asahi Techno Glass, Tokyo, Japan), and 1 μg of each transporter plasmid was transfected into subconfluent cultures of COS-1 cells using Lipofectamine 2000 (Invitrogen). The cells were used 2−3 days after transfection. They were incubated in a pretreatment buffer [140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes/Tris (pH 7.5)] with a test sample at 37 °C for 30 min. An uptake solution containing 80 mM methyl-α-D-glucopyranoside and 4 μCi/mL methyl α-D-[U−^{14}C]glucopyranoside was then added into each well, and the mixture was incubated at 37 °C for 30 min. Following incubation, the plates were washed three times with cold stop buffer [140 mM choline chloride, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Hepes/Tris (pH 7.5)] containing 300 μM phlorizin. The cells were then solubilized with 0.1 M NaOH, and their radioactivity was measured with a liquid scintillation counter (3100 TR, Perkin-Elmer). Phlorizin was used as positive control, and its IC_{50} values were 0.2 μM (SGLT1) and 0.1 μM (SGLT2), respectively.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of **1**−**4** are available free of charge via the Internet at http://pubs.acs.org.

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(27) CCDC 747369 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge at via http://www.ccdc.cam.ac.uk/deposit or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336 033; e-mail: deposit@ccdc.cam.ac.uk).

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