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SYNTHESIS AND EVALUATION OF INFLUENZA VIRUS SIALIDASE INHIBITORY ACTIVITY OF HINOKIFLAVONE-SIALIC ACID CONJUGATES

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Abstract – The known biflavonoid, hinokiflavone (**1**) was isolated from the leaves of *Metasequoia glyptostroboides* Hu et Cheng and displayed influenza A and B virus sialidase inhibitory activity. The unnatural glycoconjugate, hinokiflavone-sialic acid (**8**) was synthesized and exhibited more potent inhibitory activity.

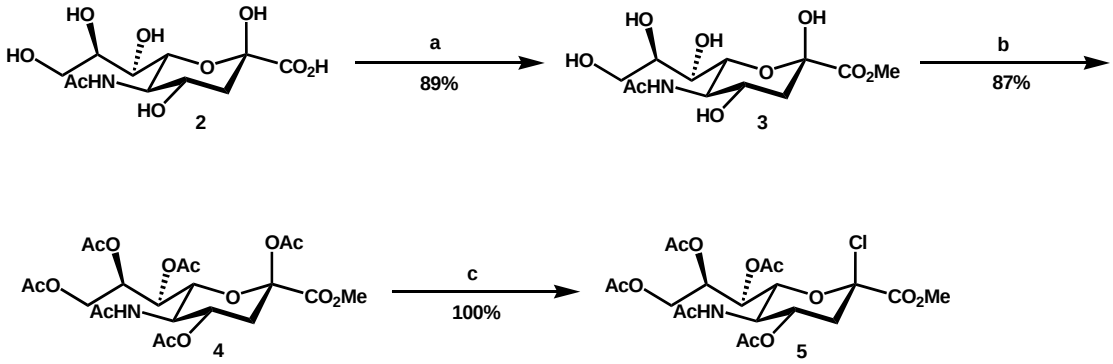
INTRODUCTION

Flavonoids are widely distributed in the plant kingdom and possess a variety of biological activities. Biflavonoids are flavonoid-dimers connected by a C-O-C or C-C bond. The biological activities of a number of biflavonoids were recently investigated and we found that a C-C bond type biflavonoid, ginkgetin, exhibits inhibitory activity against influenza virus sialidase.[1] Here we report a C-O-C type biflavonoid, hinokiflavone, which was isolated from *Metasequoia glyptostroboides* Hu et Cheng and shows inhibitory activity against influenza virus sialidase. As sialic acids are essential components of the host-cell surface receptors recognized by influenza viruses during the first stage of infection, synthetic sialic acid-aglycone conjugates might inhibit the virus. Therefore we synthesized a variety of

hinokiflavone-sialic acid conjugates and evaluated their inhibitory activity against influenza virus sialidase.

RESULTS AND DISCUSSION

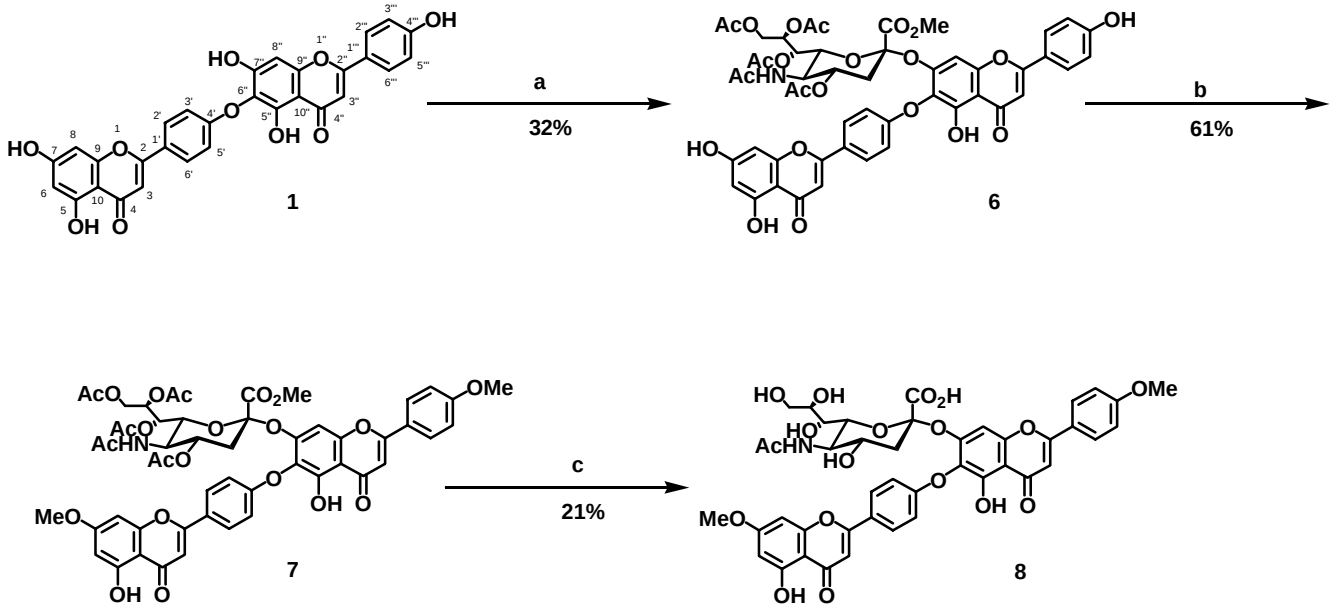
The leaves of *Metasequoia glyptostroboides* Hu et Cheng (289.4 g), collected in Tokyo prefecture, Japan, were extracted with acetone seven times. The acetone extract (26.2 g) was subjected to silica gel column chromatography using $CHCl_3$-MeOH-DMSO to obtain known biflavonoid, hinokiflavone (**1**) (758.4 mg). Hinokiflavone (**1**) was identified by comparing its spectroscopic data with reported data.[2] The glycosylation of the arylic hydroxyl group to sialic acid (Neu5Ac) (**2**) was performed by the Williamson's method.[3,4] The preparation of the methyl ester of Neu5Ac (**3**) was carried out by using a dried strong cation-exchange resin (Dowex-50W-X2) with MeOH. Following acetylation of **3** with Ac_2O and pyridine, the chloro derivative (**5**) was prepared from **4** by AcCl. Compound **5** was identified by comparing its spectral data with that reported in the literature.[5]



Scheme 1. *Reagents and conditions*: (a) MeOH, Dowex-50W-X2, rt, 48 h; (b) Ac_2O, pyridine, rt, 20 h; (c) AcCl, rt, 48 h.

To synthesize hinokiflavone-sialic acid (**6**), a Williamson's reaction using sodium hydride was carried out to combine the chloro derivative of Neu5Ac (**5**) with the C-7'' position of **1**. Compound **6** was assigned the molecular formula of $C_{50}H_{45}NO_{22}$ by its positive HRFABMS spectral data *m/z* 1012.2524 $[M+H]^+$, (calcd 1012.2512, $C_{50}H_{45}NO_{22}$, $[M+H]^+$). The UV spectrum (MeOH) of **6** showed absorption maxima at λ_{max} nm (log ε): 209 (4.80), 269 (4.56) and 336 (4.68) nm, indicating the presence of aromatic rings. The IR spectrum exhibited the presence of hydroxyl or amino groups (3430 cm^{-1}) and carbonyl (1750, 1660, 1610 cm^{-1}) groups. The ^{1}H-NMR spectrum showed six singlet peaks at δ 1.71, 1.87, 1.91, 2.03, 2.16 and 3.68 (each 3H, s) each due to a methyl group. The last methyl group was assigned to the methyl ester. The spectrum exhibited signals in the aromatic region (δ 6.20-8.15) due to the hinokiflavone skeleton, and between δ 1.91-5.40 due to the sialic acid part. The carboxyl methyl proton at δ 3.68 showed NOESY

correlations with the hinokiflavone part at δ 7.10 (8'' position) and δ 8.04 (2' and 6' position). This NOESY correlation data suggested that Neu5Ac was linked to **1** at the C-7'' position. In order to methylate the hydroxyl groups at 7 and 4''' positions, **6** was treated with CH_2N_2 in $CHCl_3$ and DMF. Finally the *O*-acetyl and methyl ester groups of **7** were hydrolyzed with NaOMe, MeOH and H_2O to yield (**8**).



Scheme 2. *Reagents and conditions*: (a) **5**, NaH, DMF, rt, 18 h; (b) CH_2N_2, $CHCl_3$, DMF, rt, 3 h; (c) NaOMe, MeOH, H_2O, rt, 23 h.

The glycosylation of Neu5Ac by Williamson's method reacts by S_N2 reaction mechanism and only gives α-glycoside.[3,4] It was slightly synthesized a glycoconjugate which was linked to **1** at the C-7 position. It seems that C-7 glycosidic linkage is very unstable to be hydrolyzed easily. In contrast, compound **6** might be stable with its complex structure.

Table 1. Inhibition of influenza A and B virus sialidases by hinokiflavone-sialic acid conjugates

Compound	Sialidase inhibitory activity [IC_{50} (μg/mL)]		
	A/New Caledonia/20/99 (H1N1)	A/Guizhou/54/89 (H3N2)	B/Ibaraki/2/85
F36	55.3	55.0	34.0
1	41.8	47.2	24.8
6	>100	>100	>100
7	>100	>100	66.7
8	19.1	13.5	8.2

Compounds **6** and **7** both showed weak inhibitory activity against sialidase of influenza A (H1N1, H3N2) and B viruses, whereas compounds **1** and **8** exhibited significant inhibitory activity. In particular, compound **8** exhibited higher inhibitory activity than F36 (5, 7, 4'-trihydroxy-8-methoxyflavone), which was isolated from the roots of *Scutellaria baicalensis*[6] and used as a positive control (Table 1).

EXPERIMENTAL

General Experimental Procedures. Melting points were determined on a Yanaco MP. Optical rotation was measured with a HORIBA SEPA-300 polarimeter. The IR spectra were recorded with a JASCO IR Report-100 spectrophotometer, and the UV spectra were recorded with a Shimadzu UV-240 spectrophotometer. The MS spectra were measured with a JEOL JMS-700 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM-LA500 MHz and JNM-AL400 MHz spectrometers (DMSO with TMS). Column chromatography was performed using KANTO CHEMICAL silica gel 60N (63-210 μm), GE Healthcare Sephadex LH-20 and Mitsubishi Chemical DIAION HP-20. HPLC was performed using a Senshu Scientific SSC-346 PUMP and measured with a SSC-5410 UV/VIS Detector using a Senshu Pak PEGASIL-ODS column. The procedure for the influenza A and B virus sialidase inhibition assay was the same as described previously.[1]

Plant material. The leaves of *Metasequoia glyptostroboides* Hu et Cheng were collected in Meiji Pharmaceutical University, Tokyo prefecture, Japan, in December 2005.

Extraction and isolation. The dried leaves of *Metasequoia glyptostroboides* Hu et Cheng (289.4 g) were extracted with acetone (4 L × 7 times). The concentrated acetone extract (26.2 g) was subjected to silica gel column chromatography (column A; 7 × 200 mm) eluted with $CHCl_3$-MeOH (100:1→50:1→30:1→10:1), MeOH and DMSO to afford 11 fractions. Fraction A11 (1.02 g) was precipitated with MeOH and filtered, isolating hinokiflavone (1) (758.4 mg) as a solid. **Hinokiflavone (1).** Yellow powder; mp 253~258 °C; IR ν_{max} (KBr) cm^{-1}: 3200, 1660, 1610, 1500, 1460, 1365, 1290, 1250, 1175, 1100, 1030, 840; UV (MeOH) λ_{max} nm (log ε): 213 (4.57), 271 (4.36), 339 (4.42); EIMS *m/z* (rel. int.): 538 ([M]$^+$, 100), 270 (12), 257 (19), 254 (11); HREIMS *m/z* 538.0895 (calcd for $C_{30}H_{18}O_{10}$, [M]$^+$ 538.0900); ^{1}H-NMR (DMSO-d_6) δ 6.20 (d, 1H, *J* = 2.0 Hz), 6.48 (d, 1H, *J* = 2.0 Hz), 6.72 (s, 1H), 6.84 (s, 1H) , 6.84 (s, 1H), 6.94 (d, 2H, *J* = 8.7 Hz), 7.96 (d, 2H, *J* = 8.7 Hz), 7.04 (d, 2H, *J* = 9.1 Hz), 8.01 (d, 2H, *J* = 9.1 Hz), 12.88 (s, 1H), 13.20 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 94.0, 94.6, 98.9, 102.5, 103.8, 103.9, 104.1, 115.3, 115.3, 116.0, 116.0, 121.1, 124.2, 124.7, 128.3, 128.3, 128.6, 128.6, 153.1, 153.7, 157.2, 157.3, 160.6, 161.3, 161.4, 163.1, 164.1, 164.2, 181.7, 182.0.

General procedure for the preparation of 3 from 2. A solution of **2** (0.016 mol) and dried strong cation-exchange resin (Dowex-50W-X2) (5.0 g) in MeOH (200 mL) was stirred at rt under Ar gas. After the reaction mixture had been stirred for 48 h, the MeOH solution was concentrated to give **3** (89 %).

White powder; mp 170~173 °C; $[\alpha]_D$ -105.0 (*c* 0.5, MeOH); IR ν_{max} (KBr) cm^{-1}: 3350, 2940, 1740, 1640, 1550, 1430, 1370, 1010, 890; FABMS (positive) *m/z* : 324 $[M+H]^+$, 309; HRFABMS (positive) *m/z* 324.1292 (calcd for $C_{12}H_{22}NO_9$, $[M+H]^+$ 324.1295); ^{1}H-NMR (DMSO-d_6) δ 1.89 (s, 3H), 2.03 (dd, 1H, *J* = 12.6, 4.7 Hz), 3.70 (s, 3H), 3.3-6.5 (m, 13H), 8.18 (d, 1H, *J* = 8.0 Hz); ^{13}C-NMR (DMSO-d_6) δ 22.4, 48.5, 52.2, 53.0, 63.5, 65.3, 69.0, 69.5, 70.4, 94.8, 170.1, 171.9.

General procedure for the preparation of 4 from 3. To a solution of **3** (0.030 mol) in pyridine (200 mL) was added Ac_2O (2.66 mol), which was then stirred at rt under Ar gas for 20 h. The solution was concentrated in vacuo, and diluted with water and $CHCl_3$. The organic layer was separated, dried over anhydrous sodium sulfate and evaporated. The crude product was purified by column chromatography on silica gel ($CHCl_3$-isopropanol) to afford **4** (87 %). White powder; mp 80~81 °C; $[\alpha]_D$ -24.8 (*c* 1.0, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3300, 2900, 1750, 1670, 1550, 1450, 1380, 1230, 1050; FABMS (positive) *m/z* : 534 $[M+H]^+$, 474, 414; HRFABMS (positive) *m/z* 534.1818 (calcd for $C_{22}H_{32}NO_{14}$, $[M+H]^+$ 534.1823); ^{1}H-NMR (DMSO-d_6) δ 1.70 (s, 3H, α-NHCO$\underline{CH_3}$), 1.70 (s, 3H, β-NHCO$\underline{CH_3}$), 1.94-2.09 (s, 15H, α-OCO$\underline{CH_3}$), 1.94-2.13 (s, 15H, β-OCO$\underline{CH_3}$), 1.90 (m, 1H), 2.42 (dd, 1H, *J* = 13.5, 5.0 Hz, β-H-3eq), 2.66 (dd, 1H, *J* = 12.9, 5.0 Hz, α-H-3eq), 3.68 (s, 3H, β-COO$\underline{CH_3}$), 3.70 (s, 3H, α-COO$\underline{CH_3}$), 3.97 (m, 1H), 4.11 (d, 1H, *J* = 10.5 Hz), 4.26 (m, 1H), 4.95 (m, 1H), 5.05 (m, 1H), 5.20 (m, 1H), 7.81 (d, 1H, *J* = 9.3 Hz, β-NH), 7.87 (d, 1H, *J* = 9.3 Hz, α-NH); ^{13}C-NMR (DMSO-d_6) δ (α) 20.4, 20.4, 20.5, 20.5, 20.6, 22.5, 35.9, 47.5, 52.7, 61.5, 66.8, 68.6, 68.7, 72.5, 96.3, 167.6, 168.3, 168.9, 169.0, 169.1, 169.6, 169.9, δ (β) 20.4, 20.4, 20.5, 20.5, 20.6, 22.5, 35.6, 47.4, 52.8, 61.3, 66.8, 68.5, 69.1, 71.5, 96.7, 166.0, 168.0, 168.9, 169.0, 169.2, 169.7, 169.9.

General procedure for the preparation of 5 from 4. Compound **4** (1.88 mmol) was dissolved in AcCl (0.42 mol) and stirred slowly for 48 h at rt. The solution was concentrated in vacuo, and diluted with water and $CHCl_3$. The organic layer was separated and dried over anhydrous sodium sulfate. Evaporation of the $CHCl_3$ layer yielded **5** (100 %). White powder; mp 112~114 °C; $[\alpha]_D$ -28.2 (*c* 0.7, $CHCl_3$); IR ν_{max} (KBr) cm^{-1}: 3300, 2860, 1750, 1660, 1540, 1440, 1220, 1035; FABMS (positive) *m/z* : 510 $[M+H]^+$, 492, 474, 414; HRFABMS (positive) *m/z* 510.1369 (calcd for $C_{20}H_{29}NO_{12}Cl$, $[M+H]^+$ 510.1378); ^{1}H-NMR (DMSO-d_6) δ 1.72 (s, 3H), 1.96 (s, 3H), 1.99 (s, 3H), 2.01 (s, 6H), 2.32 (dd, 1H, *J* = 13.9, 11.1 Hz), 2.69 (dd, 1H, *J* = 13.9, 4.4 Hz), 3.79 (s, 3H), 4.01 (m, 2H), 4.24 (d, 1H, *J* = 12.3 Hz), 4.39 (d, 1H, *J* = 10.7 Hz), 5.06 (m, 1H), 5.17 (m, 1H), 5.32 (d, 1H, *J* = 11.9 Hz), 7.95 (d, 1H, *J* = 9.5 Hz); ^{13}C-NMR (DMSO-d_6) δ 20.4, 20.5, 20.5, 20.6, 22.6, 35.6, 45.3, 52.3, 61.6, 66.9, 68.8, 69.2, 75.9, 96.7, 169.0, 169.0, 169.1, 169.8, 169.8, 169.9.

General procedure for the preparation of 6 from 1. A solution of **1** (0.37 mmol) and NaH (0.83 mmol) in DMF (15 mL) was stirred at rt under Ar gas. After stirring the reaction mixture for 1 h, compound **5**

(1.18 mmol) was added and the mixture stirred for 17 h at rt under Ar gas. The solution was diluted with saturated aqueous NaCl and $CHCl_3$. The organic layer was separated and dried over anhydrous sodium sulfate. After the $CHCl_3$ layer was evaporated, the residue was purified by Sephadex LH-20 column chromatography eluted with MeOH to give **6** (32 %). Yellow powder; mp 194~199 °C; $[\alpha]_D^{24}$ +3.9 (*c* 0.8, MeOH); IR ν_{max} (KBr) cm^{-1}: 3430, 1750, 1660, 1610, 1450, 1360, 1290, 1240, 1170, 1030, 840; UV (MeOH) λ_{max} nm (log ε): 209 (4.80), 269 (4.56), 336 (4.68); FABMS (positive) *m/z* : 1012 $[M+H]^+$, 539, 414; HRFABMS (positive) *m/z* 1012.2524 (calcd for $C_{50}H_{46}NO_{22}$, $[M+H]^+$ 1012.2512); ^{1}H-NMR (DMSO-d_6) δ 1.71 (s, 3H), 1.87 (s, 3H), 1.91 (s, 3H), 1.91 (m, 1H), 2.03 (s, 3H), 2.16 (s, 3H), 2.40 (dd, 1H, *J* = 12.9, 4.6 Hz), 3.68 (s, 3H), 3.94 (q, 1H, *J* = 10.2 Hz), 4.10 (dd, 1H, *J* = 12.2, 6.3 Hz), 4.22 (dd, 1H, *J* = 12.2, 2.9 Hz), 4.45 (dd, 1H, *J* = 10.7, 1.5 Hz), 4.72 (ddd, 1H, *J* = 10.9, 10.9, 4.6 Hz), 5.17 (dd, 1H, *J* = 8.8, 1.5 Hz), 5.40 (m, 1H), 6.20 (d, 1H, *J* = 2.2 Hz), 6.49 (d, 1H, *J* = 2.2 Hz), 6.89 (s, 1H), 6.93 (d, 2H, *J* = 8.8 Hz), 6.99 (s, 1H), 7.07 (d, 2H, *J* = 8.8 Hz), 7.10 (s, 1H), 7.80 (d, 1H, *J* = 9.8 Hz), 8.04 (d, 2H, *J* = 8.8 Hz), 8.15 (d, 2H, *J* = 8.8 Hz), 10.55 (br. s, 1H), 10.76 (br. s, 1H), 12.87 (s, 1H), 13.23 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 20.4, 20.4, 20.5, 20.5, 22.5, 37.7, 47.6, 53.4, 62.0, 66.8, 67.7, 67.9, 73.2, 93.9, 97.3, 98.8, 100.0, 102.8, 103.7, 104.0, 107.2, 115.5, 115.5, 115.8, 115.8, 120.7, 124.5, 126.7, 128.1, 128.1, 128.9, 128.9, 151.8, 152.7, 152.9, 157.2, 160.3, 161.3, 161.4, 164.1, 162.8, 164.9, 167.0, 169.0, 169.1, 169.4, 169.4, 169.7, 181.6, 182.1.

General procedure for the preparation of 7 from 6. A solution of **6** (0.028 mmol) and CH_2N_2 (2 mL) in $CHCl_3$ (5.0 mL) and DMF (1.0 mL) was stirred at rt for 3 h. After the solution was evaporated, the residue was purified by column chromatography on silica gel ($CHCl_3$-MeOH) to afford **7** (61 %). Yellow powder; mp 160~162 °C; $[\alpha]_D^{28}$ +5.4 (*c* 0.4, MeOH); IR ν_{max} (KBr) cm^{-1}: 3420, 1750, 1660, 1620, 1500, 1290, 1230, 1120, 1040, 840; UV (MeOH) λ_{max} nm (log ε): 209 (4.85), 270 (4.61), 329 (4.74); FABMS (positive) *m/z* : 1040 $[M+H]^+$, 567; HRFABMS (positive) *m/z* 1040.2822 (calcd for $C_{52}H_{50}NO_{22}$, $[M+H]^+$ 1040.2825); ^{1}H-NMR (DMSO-d_6) δ 1.71 (s, 3H), 1.87 (s, 3H), 1.91 (s, 3H), 1.94 (m, 1H), 2.03 (s, 3H), 2.19 (s, 3H), 2.40 (dd, 1H, *J* = 12.5, 4.6 Hz), 3.69 (s, 3H), 3.87 (s, 3H), 3.87 (s, 3H), 3.94 (q, 1H, *J* = 10.2 Hz), 4.10 (dd, 1H, *J* = 12.4, 6.3 Hz), 4.21 (dd, 1H, *J* = 12.3, 3.0 Hz), 4.46 (dd, 1H, *J* = 10.6, 1.3 Hz), 4.72 (ddd, 1H, *J* = 11.0, 11.0, 4.6 Hz), 5.17 (dd, 1H, *J* = 8.9, 1.3 Hz), 5.41 (m, 1H), 6.38 (d, 1H, *J* = 2.2 Hz), 6.77 (d, 1H, *J* = 2.2 Hz), 6.95 (s, 1H), 7.08 (s, 1H), 7.09 (d, 2H, *J* = 9.0 Hz), 7.11 (s, 1H), 7.12 (d, 2H, *J* = 9.0 Hz), 7.80 (d, 1H, *J* = 9.8 Hz), 8.07 (d, 2H, *J* = 9.0 Hz), 8.27 (d, 2H, *J* = 9.0 Hz), 12.87 (s, 1H), 13.17 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ 20.4, 20.5, 20.5, 20.8, 22.6, 37.7, 47.6, 53.4, 55.5, 56.0, 61.9, 66.7, 67.6, 67.9, 73.1, 92.6, 97.1, 97.9, 99.9, 103.4, 104.1, 104.6, 107.1, 114.3, 114.3, 115.4, 115.4, 122.3, 124.2, 126.6, 128.1, 128.1, 128.5, 128.5, 151.7, 152.6, 152.8, 156.9, 160.2, 160.8, 162.3, 163.0, 164.2, 164.9, 166.8, 168.8, 168.9, 169.2, 169.2, 169.6, 181.6, 182.0.

General procedure for the preparation of 8 from 7. A solution of **7** (0.017 mmol) and NaOMe (28 % in MeOH) (4 mL) in MeOH (4 mL) was stirred at rt for 6 h under Ar gas. Then H_2O (8 mL) was added and stirred for 17 h at rt. The reaction mixture was treated with 2M HCl and the solution was evaporated. The residue was purified by column chromatography on DIAION HP-20 (H_2O→MeOH) and the solvent was evaporated. The MeOH layer was purified by ODS-HPLC (80 % MeOH) to afford **8** (21 %). Yellow powder; mp (dec.) 218 °C; $[\alpha]_D^{29}$ +24.1 (*c* 0.06, MeOH); IR ν_{max} (KBr) cm^{-1}: 3450, 1650, 1620, 1500, 1450, 1380, 1250, 1180, 1120, 1030, 840; UV (MeOH) λ_{max} nm (log ε): 212 (4.73), 271 (4.48), 333 (4.59); FABMS (negative) *m/z* : 856 $[M-H]^-$, 565; HRFABMS (negative) *m/z* 856.2087 (calcd for $C_{43}H_{38}NO_{18}$, $[M-H]^-$ 856.2089); ^{1}H-NMR (DMSO-d_6) δ 1.33 (dd, 1H, *J* = 11.6, 11.6 Hz), 1.89 (s, 3H), 2.72 (dd, 1H, *J* = 11.6, 4.8 Hz), 3.34-3.84 (m, 7H), 3.87 (s, 3H), 3.87 (s, 3H), 6.38 (d, 1H, *J* = 2.2 Hz), 6.82 (d, 1H, *J* = 2.2 Hz), 6.94 (s, 1H), 6.97 (s, 1H), 7.11 (d, 2H, *J* = 8.7 Hz), 7.14 (d, 2H, *J* = 8.7 Hz), 7.66 (s, 1H), 8.04 (d, 2H, *J* = 8.7 Hz), 8.11 (d, 2H, *J* = 8.7 Hz), 8.20 (br.s), 12.89 (s, 1H), 12.89 (s, 1H).

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REFERENCES

1. K. Miki, T. Nagai, K. Suzuki, R. Tsujimura, K. Koyama, K. Kinoshita, K. Furuhata, H. Yamada, and K. Takahashi, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 772.
2. H. Geiger and K. Markham, *Z. Naturforsch.*, 1996, **51c**, 757.
3. K. Furuhata, *Trends. Glycosci. Glycotechnol.*, 2004, **16**, 143.
4. R. Myers, R. Lee, Y. Lee, G. Thomas, L. Reynolds, and Y. Uchida, *Anal. Biochem.*, 1980, **101**, 166.
5. K. Furuhata, K. Anazawa, M. Itoh, Y. Shitori, and H. Ogura, *Chem. Pharm. Bull.*, 1986, **34**, 2725.
6. T. Nagai, Y. Miyaichi, T. Tomimori, Y. Suzuki, and H. Yamada, *Chem. Pharm. Bull.*, 1990, **38**, 1329.