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Structure Determination and Total Synthesis of a Novel Antibacterial Substance, AB0022A, Produced by a Cellular Slime Mold

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A novel antibacterial substance, AB0022A, was isolated from the cellular slime mold *Dictyostelium purpureum* K1001. It inhibited the growth of Gram-positive bacteria, and its MICs ranged from 0.39 to 50 μg/ml. Because AB0022A was a highly substituted aromatic compound, we could not determine its structure based on only its physico-chemical and spectral data. We therefore used a dehalogenated derivative from AB0022A and deduced that its structure was 1,9-dihydroxy-3,7-dimethoxy-2-hexanoyl-4,6,8-trichlorodibenzofuran. To confirm this structure, we synthesized the compound having the deduced structure. The synthetic compound was identical to naturally occurring AB0022A.

Cellular slime molds are phylogenetically unique organisms that have two stages, a vegetative amoebae stage and a reproductive fruiting body stage, in their life cycles. Spore germination produces a unicellular amoeba which feeds on bacteria. When amoebae starve, they aggregate by chemotaxis to relayed cyclic AMP signals to form fruiting bodies consisting of spores and a multicellular stalk. Because of these unique characteristics, cellular slime molds are frequently used to study differentiation in various fields of biology.

Although differentiation inducing factors (DIFs)[1], discadenine[2] and several other compounds have been reported as the growth-regulating metabolites produced by cellular slime molds, no antibacterial compound has been isolated. Because cellular slime molds have not previously been considered as a source for new compounds, we investigated their use as a possible source for antibacterial compounds. Using a screening program, we isolated a new antibacterial substance, AB0022A, from the fruiting bodies of *Dictyostelium purpureum* K1001.

In this paper, we determined the structure of AB0022A (**1**, Fig. 1). Because AB0022A was a highly substituted aromatic compound, it was impossible to determine the structure using spectral data alone. We therefore deduced its structure using one of its degradation products, as shown in Fig. 1. Finally, we synthesized **1** to confirm its structure.

Results

Identification of the Producing Strain

The cellular slime mold K1001 was isolated from a soil sample collected in Nogi-machi, Tochigi, Japan. Based on its physical properties, it was identified as *Dictyostelium purpureum* by Dr. HAGIWARA (National Science Museum, Japan).

Production and Isolation of AB0022A

Fermentation of *D. purpureum* K1001 and isolation of AB0022A were carried out as described in the experimental section. AB0022A was isolated only from fruiting bodies of strain K1001. From 770 plates (90 mm in diameter) of

Fig. 1. Structure of AB0022A (**1**).

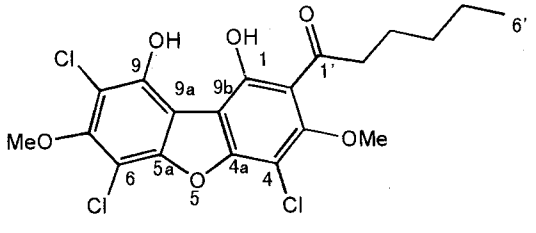


Table 1. Physico-chemical properties of AB0022A (**1**).

Appearance	Pale yellow needles
Mp	144.7–146.1 °C
IR ν max (KBr) cm^{-1}	3425, 2955, 2928, 1628, 1601, 1458, 1375, 1076
Molecular formula	$C_{20}H_{19}Cl_3O_6$
Mass spectrometry	
EI-MS (m/z)	$460(M)^+$
Positive FAB-MS (m/z)	$461(M + H)^+$
Negative FAB-MS (m/z)	$459(M - H)^-$
HR-EIMS (m/z)	Calcd. for $C_{20}H_{19}Cl_3O_6$: 460.0243
	Found: 460.0247
UV λ max ($CHCl_3$) nm (ε)	259(1990), 279(2560), 304(sh, 1130)
TLC (Rf value)	0.33 (hexane/acetone=1/1)

Table 2. Comparison of ^1H and ^{13}C NMR spectra.

^1H NMR ($CDCl_3$)	
Synthesized compound	Naturally occurring AB0022A
0.94 (3H,t,7Hz)	0.94 (3H, t, 7Hz)
1.37–1.40 (4H,m)	1.37–1.47 (4H, m)
1.77 (2H, quint., 8Hz)	1.71–1.82 (2H, m)
3.20 (2H, t, 8Hz)	3.20 (2H, t, 7Hz)
3.99 (3H, s)	3.99 (3H, s)
4.06 (3H, s)	4.06 (3H, s)
9.31 (1H, s)	9.29 (1H, s)
15.33 (1H, s)	15.33 (1H, s)

^{13}C NMR ($CDCl_3$)			
Synthesized compound		Naturally occurring AB0022A	
14.0	110.2	14.0	110.1
22.5	111.5	22.5	111.5
23.9	112.3	24.0	112.3
31.5	145.9	31.5	145.9
43.3	150.9	43.3	150.9
61.3	153.2	61.4	153.3
62.4	155.1	62.4	155.1
103.8	156.2	103.8	156.2
104.5	157.8	104.5	157.8
108.7	208.3	108.7	208.3

fruiting bodies, we obtained 4 mg of AB0022A in the form of pale-yellow, needle-shaped crystals.

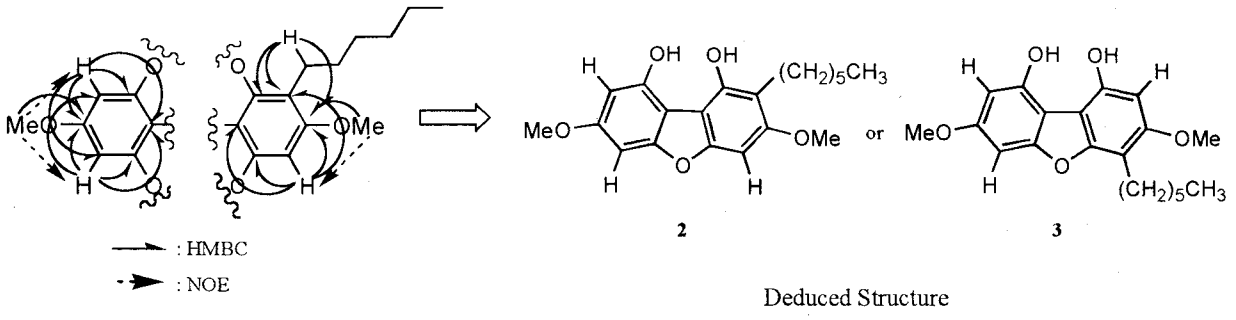
Structure Elucidation

The physico-chemical properties of AB0022A (**1**) are shown in Table 1. The molecular formula of **1** was determined as $C_{20}H_{19}Cl_3O_6$ from the EI-MS, FAB-MS and HREI-MS data. This determination was further supported by the ^1H and ^{13}C NMR spectra (Table 2).

^1H and ^{13}C NMR spectra, DEPT experiments and the molecular formula indicated the presence of one $-CH_3$, two $-OCH_3$, four $-CH_2-$, one $-C=O$, two $-OH$ and twelve non-protonated olefinic carbon atoms. ^1H-^1H COSY, HMBC and HMQC spectra showed the presence of a hexanoyl group. In spite of these experiments, the structure of **1** was not clear, because AB0022A was a fully substituted aromatic compound.

We therefore attempted dechlorination of **1** by hydrogenation. The hydrogenation of **1** on Pd/C or $Pd(OH)_2/C$ gave a complex mixture of partially or completely hydrogenated compounds. Fortunately, hydrogenation on $Pd/BaSO_4$ gave only a single product, whose molecular formula, as indicated by HR-EIMS, was $C_{20}H_{24}O_5$. Comparison between the ^1H NMR spectra of this product and of **1** revealed several changes. Three aromatic proton signals appeared: one each at 6.39, 6.66 and 6.68 ppm. The methylene signal at 3.20 ppm disappeared, and two methylene signals, one at 1.21~1.44 and one at 2.70 ppm, appeared. The appearances of the three aromatic

Fig. 2. Structure elucidation of the dehalogenated derivative.



protons suggested that three chlorine atoms of **1** were removed. In addition, the changes of the methylene signals indicated that a carbonyl group of **1** was reduced. These results were supported by those of the DEPT experiments. Thus the HMQC, HMBC and DIF-NOE experiments revealed the partial structure of the hydrogenated compound (Fig. 2); based on the molecular formula, we were then able to deduce that the hydrogenated compound had a structure of **2** or **3**.

In the ^{1}H NMR spectrum of **1**, we observed a 1-OH proton signal at 15.3 ppm. This suggested that the 1-OH proton formed a hydrogen-bond with the oxygen of the hexanoyl group. From this fact and structure determination studies on the hydrogenated derivative, we deduced that the structure of **1** was 1,9-dihydroxy-3,7-dimethoxy-2-hexanoyl-4,6,8-trichlorodibenzofuran (Fig. 1), and also that the structure of the dehalogenated derivative was **2**.

Total Synthesis of AB0022A

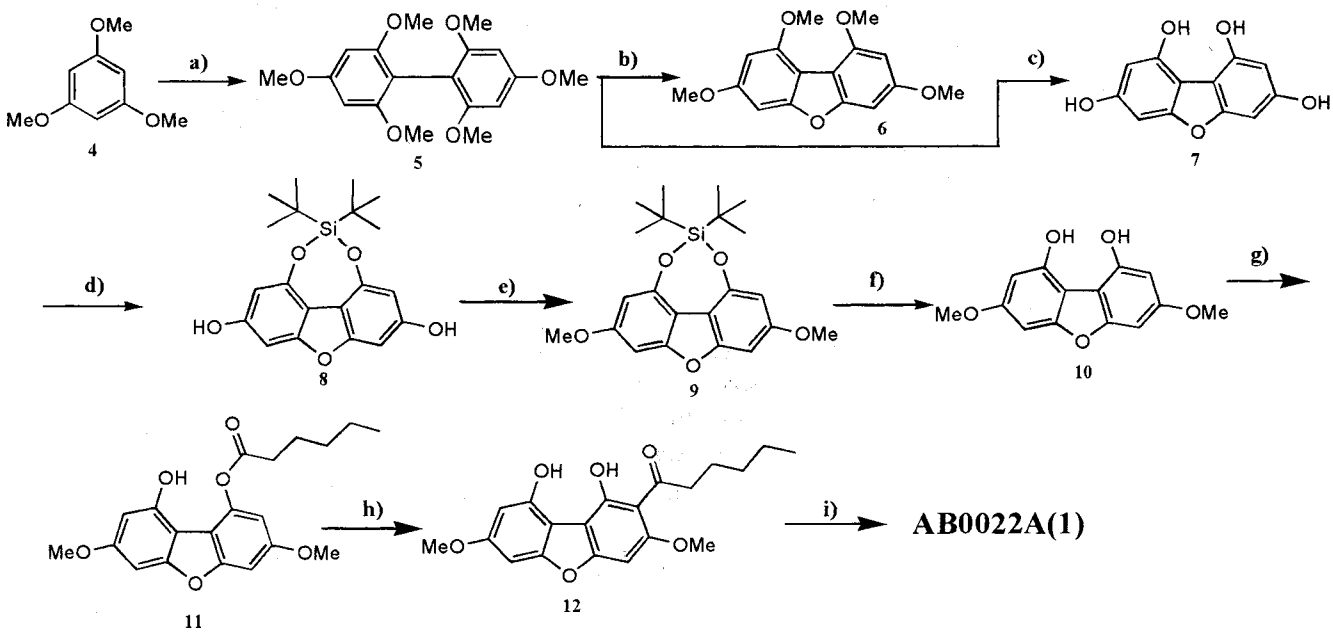
The strategy for synthesizing AB0022A (**1**) was as follows: we selected 1,3,7,9-tetramethoxydibenzofuran (**6**), which is known to be synthesized from 1,3,5-trimethoxybenzene (**4**) in three steps[3], as a starting material; 1,3,7,9-tetrahydroxydibenzofuran (**7**), which was a suitable intermediate for the total synthesis of **1**, could be easily derived from **6**; a selective methylation at the 3- and 7-hydroxy groups, an acylation at the C-2 position, and a successive chlorination would give **1**.

At first, we tried to synthesize 1,3,7,9-tetramethoxydibenzofuran (**6**) according to the reported procedures[3]. Iodination of 1,3,5-trimethoxybenzene (**4**) and Ullmann coupling gave 2,2′,4,4′,6,6′-hexamethoxybiphenyl (**5**). Cyclization of this biphenyl under the reported condition (57% HI aq., reflux, 0.5 hours)[3] gave a complex mixture, which was methylated with iodomethane to give 1,3,7,9-tetramethoxydibenzofuran (**6**) in low yield (12%, lit.[3] 37%). Because the reaction mixture under the reported conditions was in suspension, partial cyclization and demethylation occurred to give a complex mixture. We thought that addition of co-solvent and elongation of the reaction time would complete the reaction. We therefore modified the condition as shown in Scheme 1 (57% HI aq., AcOH, reflux, 15 hours), to directly give the desired key intermediate (**7**) in good yield (84%).

Next, we tried selective methylation at the 3- and 7-hydroxy groups of **7**. Direct methylation with two equivalent iodomethane or dimethylsulfate gave an inseparable mixture. We then attempted to protect the 1,9-hydroxy groups with a cyclic protective group. The di-*tert*-butylsilyl group[4], which is a selective protective group for 1,3- or 1,4-diol, could be easily introduced to the 1- and 9-hydroxy groups of **7** to give a silylated compound (**8**). Methylation of **8** and successive deprotection under standard conditions gave the desired 1,9-dihydroxy-3,7-dimethoxydibenzofuran (**10**) in good yield (64% in three steps).

The next step was the selective introduction of the acyl side chain to the C-2 position of **10**. Friedel-Crafts acylation of **9** ($SnCl_4$, CH_2Cl_2, r.t., 3 hours) gave exclusively an undesired 4-acyl derivative (data not shown). We therefore carried out a Fries rearrangement of the 1-*O*-acyl derivative (**11**). Fries rearrangement promoted with Lewis acid ($AlCl_3$, CH_2Cl_2, r.t., 1 hour) succeeded in giving the desired 2-acyl derivative, the yield, however, was very low; the main product was a deacylated compound.

Scheme 1. Total synthesis of AB0022A.



a) i) HIO_3, I_2, EtOH, reflux, 30 min, 91%, ii) Cu, 240°C, 6 hr, 81%; b) 57% HIaq, reflux, 30 min, then CH_3I, K_2CO_3, DMF, r.t., 3 hr, 12% (lit. 37%[3]); c) 57% HI aq., AcOH, reflux, 15 hr, 84%; d) (*tert*-Butyl)$_2$Si(OTf)$_2$, 2,6-lutidine, DMF, 0°C, 30 min, 67%; e) CH_3I, K_2CO_3, DMF, r.t., 4 hr, 97%; f) tetra-*n*-butylammonium fluoride, THF, r.t., 30 min, 94%.; g) caproyl chloride, pyridine, CH_2Cl_2, 0°C, 30 min, 66%; h) hv, benzene, r.t., 4 hr, 40%; i) $Bn(Me)_3N^+ICl_4^-$, AcOH, r.t., 3 hr, 41%.

Elemental analysis of **11** suggested that this compound captured a molecule of water. We thought that this was the reason why the yield of Fries rearrangement with Lewis acid was very low. In order to avoid the deacylation, we carried out a photo-induced Fries rearrangement[5], which proceed *via* a radical mechanism. The photo-induced Fries rearrangement of **11** with a high pressure mercury lamp gave the 2-acyl derivative (**12**) in moderate yield (40%). The structure of **12** was confirmed by HMBC NMR spectra.

Finally, we tried chlorination of **12**. Normal chlorination methods using chlorine gas or sulfuryl chloride failed to give AB0022A (**1**). However, chlorination with benzyltrimethylammonium tetrachloroiodate[6] gave **1** in moderate yield (41%).

The ^1H and ^{13}C NMR spectra of the synthetic product were identical to those of the naturally occurring **1** (Table 2).

Biological Activities

The antibacterial activities of AB0022A (**1**) are shown in Table 3. Compound **1** inhibited the growth of Gram-positive bacteria. The MICs ranged from 0.39 to 50 μg/ml. However, **1** did not inhibit the growth of Gram-negative bacteria at 100 μg/ml.

Discussion

In our continuing search for antibacteral substances from a cellular slime mold, we obtained a novel antibacterial AB0022A (**1**). This was the first reported isolation of an antibacterial metabolite from a cellular slime mold. Although cellular slime molds had not yet been used as a source for antibacterial substances, our result suggests that cellular slime molds might be good sources for new antibacterial substances.

However, because **1** was a fully substituted aromatic

Table 3. Antibacterial activities of AB0022A.

Tested organism	MIC (μg/mL)
Batillus subtilis ATCC6633	0.78
Staphylococcus aureus Smith	0.78
S. aureus IID671	1.56
S. aureus IID1677 (MRSA)	3.13
S. epidermidis IID866	0.39
Streptococcus pneumoniae IID553	25
S. pneumoniae Type III	25
S. pyogenes IID689	25
Enterococcus faecalis ATCC29212	50
E. faecium GIFU8355	50
Micrococcus luteus ATCC9431	12.5
Escherichia coli ATCC8739	>100
E. coli ATCC25922	>100
Citrobacter freundii IID976	>100
Shigella sonnei IID969	>100
Klebsiella pneumoniae IID5209	>100
Enterobacter cloacae IID977	>100
Serratia marcescens IID5218	>100
Pseudomonas aeruginosa ATCC9027	>100

compound, we were unable to determine its structure based on only its spectral data. We therefore hydrogenated **1** in order to determine the structure. Fortunately, hydrogenation gave a dehalogenated and deoxygenated compound **2**. From the spectral data of **2**, we could easily determine its partial structure. This partial structure and the ^{1}H NMR spectrum of AB0022A suggested that the structure of **1** was 1,9-dihydroxy-3,7-dimethoxy-2-hexanoyl-4,6,8-trichlorodibenzofuran.

In order to confirm this structure, we carried out the total synthesis of **1**. There were two key problems to be overcome in this synthesis: selective methylation at 3,7-OH and selective introduction of a 2-hexanoyl group. The former could easily be overcome by protection of 1,9-OH with a di-*tert*-butylsilyl group. On the other hand, the latter presented several difficulties. Friedel-crafts acylation exclusively gave an undesired 4-acylated compound; we therefore carried out a Fries rearrangement. In this way, we were able to obtain the desired 2-acylated compound with the rearrangement of **11**, however the main product was a deacylated compound (**10**). We considered that the low yield was due both to the capture of water and to deactivation of the aromatic ring with Lewis acid. Hence we carried out a photo-induced Fries rearrangement. In this way, we were able to obtain the desired compound in moderate yield. Chlorination of **12** then gave **1** which was identical with the natural product. From these results, we determined that the structure of AB0022A was 1,9-dihydroxy-3,7-dimethoxy-2-hexanoyl-4,6,8-trichlorodibenzofuran. There were a few reports of naturally occurring 1,3,7,9-tetrahydroxydibenzofuran derivatives, such as rhodomyrtoxins[3]. Our studies revealed that **1** had a unique substitution pattern on a dibenzofuran ring.

Importantly the synthesis was quite short and thereby useful for syntheses of many derivatives. In fact, we successfully synthesized a 2-acetyl, a 2-valeryl and a 2-benzoyl derivative *via* this route. The details of these other syntheses will be reported elsewhere.

AB0022A (**1**) is generally isolated from fruiting bodies of the cellular slime mold K1001. The isolation yield of **1** however is very low, due to the difficulty of fermentation of fruiting bodies. The present synthesis might enable the bulk supply of **1**. Further pharmacological and biological studies are currently underway in our laboratory.

Experimental

General

The melting point was obtained using a Yanako MP-500D micro melting point apparatus. IR spectra were recorded on a JASCO FT/IR-5300 infrared spectrophotometer. Mass spectra were obtained using a JEOL JMS-SX102A mass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EX400 NMR spectrometer. All chemical shifts were given in ppm relative to TMS as an internal standard. Ultraviolet spectra were measured on a Hitachi U-3210 spectrophotometer.

Isolation of the Strain K1001

The producing organism, cellular slime mold strain K1001, was isolated from a soil sample collected in Nogi-machi, Tochigi, Japan. A suspension of one gram of soil in 3 ml sterile water was filtered through sterile cotton gauze, and then the filtrate was mixed with an overnight culture of *Escherichia coli* NIHJ JC-2. This mixture was inoculated on a non-nutrient agar plate (KH_2PO_4 1.45%, $NaH_2PO_4 \cdot 12H_2O$ 2.40%, agar 2.00%, pH 6.41), which plate was incubated at 22°C under light from a 10 W fluorescent lamp. After 7 days, fruiting bodies of a cellular slime mold were isolated from this cultivation plate.

Fermentation

Spores of strain K1001 were suspended in sterilized

Bonner's salt solution (NaCl 0.060%, KCl 0.075%, $CaCl_2 \cdot 2H_2O$, 0.034%). To this suspension was added an overnight culture of *E. coli* NIHJ JC-2 grown in A medium (glucose 0.5%, polypepton 0.5%, yeast extract 0.05%, KH_2PO_4 0.225%, $Na_2HPO_4 \cdot 12H_2O$ 0.137%, $MgSO_4 \cdot 7H_2O$ 0.05%, pH 6.28) at the same volume as the suspension. 200 μl of the mixture was inoculated on A agar plates (A medium solidified with 1.5% agar, 90 mm in diameter), which plates were incubated for 10 days at 22°C under light from a 10 W fluorescent lamp.

Isolation of AB0022A

The fruiting bodies from approximately 100 plates of *D. purpureum* K1001 were collected and suspended in distilled water. After lyophilization, the residue was extracted with 90% methanol (40 ml) overnight at 4°C. Filtration of the extract and successive concentration gave an aqueous residue, to which was added distilled water (20 ml) and saturated NaCl (20 ml). Then the mixture was extracted with EtOAc (50 ml×3), and evaporated to give an EtOAc-soluble substance. This extraction process was repeated 7 times, and 540 mg of an EtOAc-soluble substance was obtained from 770 plates.

To a solution of this substance in acetonitrile (560 ml) was added 0.2 M acetic acid (140 ml), and then the mixture was extracted with hexane (700 ml×3). The hexane extract was re-extracted three times with 700 ml of acetonitrile-0.1% NH_4OH aq. (8 : 2). After concentration of the alkaline acetonitrile extract, the residue was supplemented with saturated NaCl (50 ml), and the mixture was extracted three times with EtOAc (300 ml). Concentration of the extract gave 83 mg of an active substance containing AB0022A.

The active substance was purified using middle pressure liquid chromatography (silica gel 60H, Merck, column size=2.2×40 cm, hexane : acetone=6 : 4, 15 ml/minute, 10 ml/fraction). Concentration of active fractions gave 18 mg of an active substance, which was further purified using a Sep-Pak ODS cartridge (Sep-Pak Vac C-18 cartridge (1 g), Waters, acetonitrile : 0.1% NH_4OH aq.=8 : 2, 1 ml/fraction). After concentration of active fractions, the residue was dissolved in 4 ml acetonitrile, and then supplemented with 0.2 M acetic acid (1 ml). This mixture was left undisturbed for 1.5 hours at room temperature to give pale-yellow, needle-shaped crystals of AB0022A (4 mg).

Antibacterial Activity

Antibacterial activity of AB0022A was assayed using paper-disk diffusion methods against *Bacillus subtilis*. The MICs of AB0022A against bacteria were determined by the two-fold agar dilution method in Mueller-Hinton Medium (Difco, Detroit, MI) as recommended by the Japan Society of Chemotherapy[7].

Hydrogenation of AB0022A (**1**)

To a solution of **1** (1.00 mg) in 1 ml of methanol was added $Pd/BaSO_4$ (100 mg). The mixture was stirred under H_2 at room temperature. After 3 hours the reaction mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was purified by silica-gel column chromatography (9 : 1 hexane : EtOAc) to give **2** as a pale purple solid (0.59 mg, 79%); ^{1}H NMR ($CDCl_3$): 0.89 (3H, t, J=7.3 Hz, 6′-CH_3), 1.21~1.44 (6H, m, 3′,4′,5′-CH_2–), 1.49~1.70 (2H, m, 2′-CH_2–), 2.70 (2H, t, J=7.6 Hz, 1′-CH_2–), 3.85 (3H, s, 7-OMe), 3.87 (3H, s, 3-OMe), 6.39 (1H, d, J=2.0 Hz, 8-H), 6.66 (1H, d, J=2.0 Hz, 6-H), 6.68 (1H, s, 4-H); ^{13}C NMR ($CDCl_3$): 14.1 (C-6′), 22.7 (C-5′), 23.2 (C-1′), 29.5 (C-2′), 29.7 (C-3′), 31.8 (C-4′), 55.9 (7-OMe), 56.1 (3-OMe), 88.0 (C-4), 89.7 (C-6), 96.8 (C-8), 104.9 (C-9b), 105.6 (C-9a), 111.5 (C-2), 146.2 (C-1), 148.8 (C-9), 155.3 (C-4a), 157.6 (C-3), 157.6 (C-5a), 159.7 (C-7); EI-MS m/z 344 $(M)^+$; HREI-MS, calcd for $C_{20}H_{24}O_5$, 344.1624, found 344.1605.

2,4,6-Trimethoxyiodobenzene and 2,2′,4,4′,6,6′-Hexamethoxybiphenyl (**5**)

2,4,6-Trimethoxyiodobenzene[8] and 2,2′,4,4′,6,6′,-hexamethoxybiphenyl[3] were prepared according to the previously described procedures.

1,3,7,9-Tetrahydroxydibenzofuran (**7**)

A mixture of **5** (1.00 g, 2.99 mmol) and acetic acid (10 ml) in 57% HI aq. (10 ml) was heated under reflux for 15 hours. After cooling, the mixture was poured into 1% $NaHSO_3$ aq. and extracted with EtOAc. The organic layer was washed with water and brine, and dried over Na_2SO_4. After evaporation, the residue was purified by silica-gel column chromatography (1 : 1 hexane : EtOAc) to give **5** as a pale brown solid (581 mg, 84%); ^{1}H NMR (DMSO-d_6): 6.20 (2H, s), 6.41 (2H, s), 9.54 (2H, brs), 10.36 (2H, brs); EI-MS m/z 232 $(M)^+$; HREI-MS, calcd for $C_{12}H_8O_5$ 232.0372, found 232.0356.

1,9-Dihydroxy-3,7-dimethoxydibenzofuran (**10**)

To a mixture of **7** (1.00 g, 4.31 mmol) and 2,6-lutidine (1.50 ml, 12.9 mmol) in dry DMF (20 ml) was added dropwise di-*tert*-butylsilyl bis(trifluoromethanesulfonate) (1.90 g, 4.31 mmol) in dry DMF (5 ml) at 0°C. After stirring at 0°C for 0.5 hours, the reaction mixture was poured into water and extracted with EtOAc. The organic

layer was washed with water and brine, and dried over Na_2SO_4. After evaporation, the residue was purified on the silica gel (3 : 1 hexane : EtOAc) to give **8** as a white solid (1.07 g, 67%); MP 249.0~250.5°C; ^{1}H NMR (DMSO-d_6): 1.03 (18H, s), 6.32 (2H, d, J=2.0 Hz), 6.58 (2H, d, J=2.0 Hz), 9.76 (2H, brs); EI-MS m/z 372 $(M)^+$; HREI-MS, calcd for $C_{20}H_{24}O_5Si$ 372.1393, found 372.1388.

To a mixture of **8** (1.07 g, 2.87 mmol) and K_2CO_3 (2.10 g, 15.2 mmol) in dry DMF (25 ml) was added iodomethane (0.70 ml, 11.3 mmol) at r.t.. After stirring for 4 hours at r.t., the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, and dried over Na_2SO_4. After evaporation, the residue was purified over silica-gel (from hexane to 10 : 1 hexane : EtOAc) to give a white solid of **9** (1.11 g, 97%); MP 151.0~153.0°C; ^{1}H NMR ($CDCl_3$): 1.08 (18H, s), 3.86 (6H, s), 6.47 (2H, d, J=2.0 Hz), 6.68 (2H, d, J=2.0 Hz); EI-MS m/z 400 $(M)^+$; *Anal* calcd for $C_{22}H_{28}O_5Si$ C: 65.97%, H: 7.05%, found C: 65.88%, H: 7.03%.

To the solution of **9** (100 mg, 0.25 mmol) in THF (1.5 ml) was added tetra-*n*-butylammonium fluoride (1 mol/liter in THF, 1.5 ml), and the mixture was stirred for 0.5 hours at r.t.. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water and brine, and dried over Na_2SO_4. After evaporation, the residue was purified by silica-gel column chromatography (3 : 1 hexane : EtOAc) to give crude **10** as a white solid (61 mg, 94%). This could be recrystallized with CH_2Cl_2; MP 172.0~172.5°C; ^{1}H NMR ($CDCl_3$) 3.85 (6H, s), 6.39 (2H, d, J=2.0 Hz), 6.67 (2H, d, J=2.0 Hz); EI-MS m/z 260 $(M)^+$; HREI-MS, calcd. for $C_{14}H_{12}O_5$ 260.0685, found 260.0686.

3,7-Dimethoxy-1-hexanoyloxy-9-hydroxydibenzofuran (**11**)

To a solution of **10** (500 mg, 1.92 mmol) and pyridine (0.50 ml) in 10 ml of CH_2Cl_2 was slowly added caproyl chloride (0.28 ml, 2.00 mmol) at 0°C. After stirring at r.t. for 0.5 hours, the reaction mixture was poured into diluted HCl aq. and extracted with EtOAc. The organic layer was washed with water and brine, and dried over Na_2SO_4. After evaporation, the residue was purified by silica-gel column chromatography (5 : 1 hexane : EtOAc) to give a white crystal **11** (monohydrate, 476 mg, 66%); MP 111.0~113.0°C; ^{1}H NMR ($CDCl_3$): 0.94 (3H, t, J=7.0 Hz), 1.37~1.45 (4H, m), 1.82 (2H, quint, J=7.0 Hz), 2.70 (2H, t, J=7.0 Hz), 3.86 (3H, s), 3.88 (3H, s), 6.42 (1H, d, J=2.0 Hz), 6.65 (1H, d, J=2.0 Hz), 6.93 (1H, d, J=2.0 Hz), 6.94 (1H, d, J=2.0 Hz), 7.14 (1H, s); EI-MS m/z 358 $(M)^+$; *Anal* calcd for $C_{20}H_{22}O_6 \cdot H_2O$ C: 63.82%, H: 6.43%, found C: 63.82%, H: 6.42%.

1,9-Dihydroxy-3,7-dimethoxy-2-hexanoyldibenzofuran (**12**)

A solution of **11** (320 mg, 0.85 mmol) in dry benzene, which had been purged with Ar for 1 hour, was irradiated at r.t. for 4 hours with a 100 W high pressure mercury lamp through a quartz jacket. After irradiation, the reaction mixture was evaporated, and the residue was purified on silica-gel (5 : 1 hexane : EtOAc) to give **12** as a pale yellow solid (122 mg, 40%); MP 143.0~144.4°C; ^{1}H NMR ($CDCl_3$): 0.93 (3H, t, J=7.0 Hz), 1.38 (4H, m), 1.72 (2H, quint., J=7.0 Hz), 3.10 (2H, t, J=7.0 Hz), 3.85 (3H, s), 3.98 (3H, s), 6.46 (1H, d, J=2.0 Hz), 6.60 (1H, s), 6.61 (1H, d, J=2.0 Hz), 8.74 (1H, s), 15.66 (1H, s); EI-MS m/z 358 $(M)^+$; *Anal* calcd for $C_{20}H_{22}O_6$ C: 67.03%, H: 6.19%, found C: 66.97%, H: 6.19%.

1,9-Dihydroxy-3,7-dimethoxy-2-hexanoyl-4,6,8-trichlorodibenzofuran (AB0022A, **1**)

To a solution of **12** (122 mg, 0.34 mmol) in acetic acid (20 ml) was added benzyltrimethylammonium tetrachloroiodate (520 mg, 1.24 mmol) under Ar. The mixture was dissolved by heating, then stirred for 3 hours at r.t.. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water, $NaHSO_3$ aq. and brine, and then dried over Na_2SO_4. After evaporation, the residue was purified by silica-gel column chromatography (3 : 1 hexane : EtOAc) to give AB0022A as a dark yellow solid (65 mg, 41%) which was insoluble to $CDCl_3$. This solid was recrystallized, according to the procedure for a naturally occurring AB0022A, to give a yellow crystal soluble to $CDCl_3$. All spectral data of the synthetic product were identical to those of the naturally occurring AB0022A.

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