A Cytotoxic Constituent of *Lysimachia japonica* THUNB. (Primulaceae) and the Structure–Activity Relationships of Related Compounds

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A cytotoxic alkylresorcinol was isolated from *Lysimachia japonica* THUNB. (Primulaceae) and identified as grevillol (2). It was tested for cytotoxicity against KB, B-16, PC-13, L-5178Y, P-388, and HEp-2 cells *in vitro*. Synthetic related compounds were also tested for cytotoxicity against the KB cell line, and the structure–activity relationships are discussed.

Keywords *Lysimachia japonica*; cytotoxicity; grevillol; 5-alkylresorcinol; structure–activity relationship

Lysimachia japonica THUNB. (Primulaceae) is a perennial herbaceous plant that grows wild on hills and mountainsides. The plant has been used as a folk medicine for treatment of boils, swellings, and rashes in Japan. Salicylic acid and some common flavonoids have been isolated from it.[1] Investigation of the biologically active constituents of this plant resulted in the isolation of 6-tridecylresorcylic acid (**1**) and grevillol (**2**) Na^+-K^+ adenosine triphosphatase (ATPase) inhibitors.[2]

In this continuation of our research on cytotoxic compounds in the plants, we found that the MeOH extract of the fresh plant markedly inhibited the growth of cultured KB cells. In this paper we report the isolation and identification of a cytotoxic principle of this plant and its cytotoxic activities against several lines of cancer cells in culture, and we also discuss the structure–activity relationship of related compounds.

The MeOH extract was partitioned between $CHCl_3$ and H_2O, and the $CHCl_3$ extract was partitioned between 95% MeOH and petroleum benzin (Fig. 1). The 95% MeOH extract, which was most active (Table I), was applied to a silica gel column and eluted with $CHCl_3$–MeOH mixture. The most active fraction eluted from the column was rechromatographed on a silica gel column to afford six crystalline compounds, **1**—**6**. Commpounds **1** and **2** were resorcinol derivatives and they were identified as 6-tridecylresorcylic acid (**1**) and grevillol (**2**) by comparison of the physical and spectral data with those of authentic samples. Compounds **3** and **4** gave positive reduction tests for flavonoids and they were identified as kaempferol (**3**) and quercetin (**4**) by comparison of the spectral data with those of authentic samples. Compounds **5** and **6** gave positive Liebermann-Burchard tests for phytosterols and they were identified as β-sitosterol (**5**) and β-sitosterol-β-D-glucoside (**6**) by direct comparison with authentic samples.

Compound **2** exhibited the highest activity among compounds **1**—**6** isolated from the most active fraction. The diacetyl derivative (**7**) and dimethyl ether (**8**) of **2** were inactive against KB cells. However, some alkylresorcinol derivatives inhibited spindle formation of fertilized sea urchin embryo.[3,4] Therefore, it appeared to be of interest to investigate the structure–cytotoxicity relationships among grevillol analogs against KB cells. Various analogs were synthesized according to route 1 or 2 in Chart 1 and their cytotoxicities were tested against KB cells *in vitro*. The results are shown in Tables II and III. Both acetylated and methylated derivatives were inactive. Compounds **9**—**14**,

TABLE I. Cytotoxicities of Extracts and Isolated Compounds toward KB Cells

Extracts	Growth inhibition (%) at 25 μg/ml	Isolated compounds	ED_{50} (μg/ml)
MeOH	98[a]	**1**	79
H_2O	0	**2**	1.1
$CHCl_3$	65	**3**	>100
95% MeOH	91	**4**	>100
PB	15	**5**	>100
		6	>100

a) At 50 μg/ml.

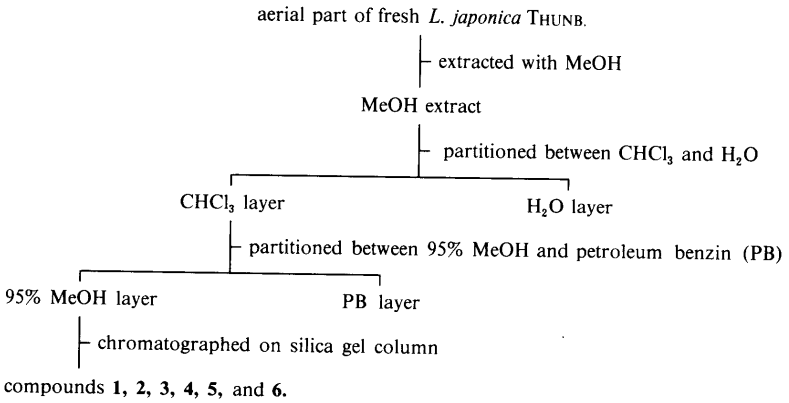


Fig. 1. Extraction and Separation of *Lysimachia japonica* THUNB.

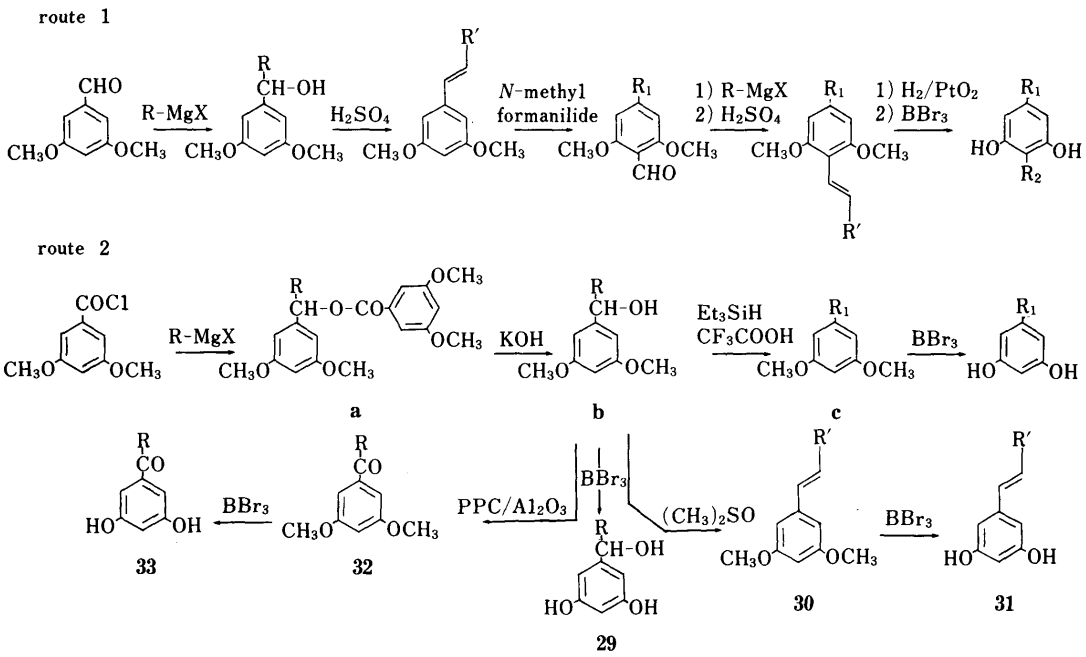
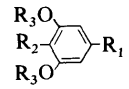


Chart 1

TABLE II. Cytotoxicities of Resorcinol Derivatives toward KB Cells



Compd.	R_1	R_2	R_3	ED_{50} (μg/ml)
7	$(CH_2)_{12}$–CH_3	H	Ac	>100
8	$(CH_2)_{12}$–CH_3	H	CH_3	>100
9	$(CH_2)_3$–CH_3	$(CH_2)_5$–CH_3	H	>100
10	$(CH_2)_5$–CH_3	$(CH_2)_5$–CH_3	H	>100
11	$(CH_2)_2$–CH_3	$(CH_2)_4$–CH_3	H	20
12	$(CH_2)_2$–CH_3	$(CH_2)_3$–CH_3	H	8.5
13	CH_3	$(CH_2)_5$–CH_3	H	24
14	H	$(CH_2)_5$–CH_3	H	25
15	$(CH_2)_4$–CH_3	H	H	19
16	$(CH_2)_5$–CH_3	H	H	15
17	CH_3	H	H	>100
18	CH_2–CH_3	H	H	>100
19	$(CH_2)_6$–CH_3	H	H	5.0
20	$(CH_2)_8$–CH_3	H	H	3.1
21	$(CH_2)_{10}$–CH_3	H	H	2.4
2	$(CH_2)_{12}$–CH_3	H	H	1.1
22	$(CH_2)_{14}$–CH_3	H	H	1.6
23	$(CH_2)_{16}$–CH_3	H	H	4.5
24	$(CH_2)_{18}$–CH_3	H	H	>50
25	$(CH_2)_{10}$–CH_3	H	Ac	>100
26	$(CH_2)_{10}$–CH_3	H	CH_3	>100
27	$(CH_2)_{14}$–CH_3	H	Ac	>100
28	$(CH_2)_{14}$–CH_3	H	CH_3	>100
22a	$CH(OCO$–$C_6H_3(OMe)_2)$–$(CH_2)_{13}$–CH_3	H	CH_3	>100
29	$CH(OH)$–$(CH_2)_{13}$–CH_3	H	H	1.8
30	$CH=CH$–$(CH_2)_{13}$–CH_3	H	CH_3	>100
31	$CH=CH$–$(CH_2)_{12}$–CH_3	H	H	0.4
32	CO–$(CH_2)_{13}$–CH_3	H	CH_3	>100
33	CO–$(CH_2)_{13}$–CH_3	H	H	0.8

TABLE III. Cytotoxicities of 5-Alkylresorcinol Derivatives toward KB Cells

Compd.	Carbon number of side chain	ED_{50} (M)
17	1	
18	2	
15	5	1.1×10^{-4}
19	7	2.4×10^{-5}
20	9	1.3×10^{-5}
21	11	9.1×10^{-6}
2	13	3.8×10^{-6}
22	15	5.0×10^{-6}
23	17	1.3×10^{-5}
24	19	

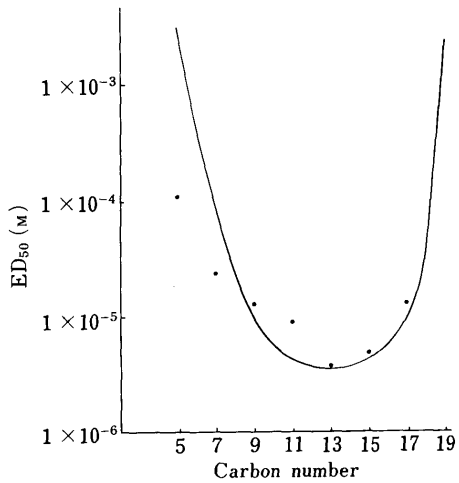


Fig. 2. Relationship Between Cytotoxicity to KB Cells *in vitro* and Carbon Number of Side Chain of 5-Alkylresorcinol Derivatives

which inhibit the spindle formation of fertilized sea urchin eggs,[3,4] were also inactive. Among 5-alkylresorcinol derivatives, **2**, **20**, **21**, and **22** showed significant cytotoxic activities with ED_{50} values of 1.1, 3.1, 2.4, and 1.6 μg/ml, respectively. A comparison of the cytotoxicities of **2** and **8**—**28** indicated that compounds with free hydroxyl groups on the benzene ring and a certain length of side chain at C-5, such as compounds **2** and **20**—**22**, showed potent

TABLE IV. Cytotoxicities of **2** toward Cancer Cell Lines (*in Vitro*, ED_{50} (μg/ml))

Compound	B-16	PC-13	L-5178Y	P-388	HEp-2
2	4.0	4.3	>25	>25	15

cytotoxicity, while acetylated and methylated derivatives, such as compounds **7**, **8**, and **25**—**28**, were inactive. Based upon the above data, it is concluded that hydroxylation at C-1 and C-3 and a certain carbon number in the side chain at C-5 are the structural requirements for potent cytotoxicity toward KB cells. The relationship between the carbon number and the cytotoxicity is also shown in Fig. 2. Compound **2** is the most active in this system. Compound **2** was also tested against several cancer cell lines *in vitro*, and the results are shown in Table IV. The compound exhibited cytotoxicity to B-16 and PC-13 cells, but was inactive against L-5178Y, P-388, and HEp-2 cells. It also appeared to be of interest to investigate the cytotoxicity of an intermediate **22a**, and compounds **29**—**33** were derived from **22a**. Among them, **29**, **31**, and **33** showed significant cytotoxicities, and compounds **31** and **33** were more active than compound **22**. It seems that an unsaturated side chain conjugated with the aromatic ring results in greater cytotoxicity than a saturated chain in this series.

Experimental

General Procedures All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Ultraviolet (UV) spectra were determined on a Hitachi 220 S double beam spectrophotometer and infrared (IR) spectra were taken on a Hitachi 260-10 infrared spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken with a JEOL JNM-GX270 (at 270 MHz) spectrometer and chemical shifts are given in δ (ppm) values referred to internal tetramethylsilane (TMS). The following abbreviations are used: br s = broad singlet, d = doublet, t = triplet, br t = broad triplet, dd = doublet of doublets, dt = doublet of triplets. Mass spectra (MS) were obtained with a JEOL JMS-D-200 mass spectrometer operating at 70 eV.

Plant Material Aerial parts of *Lysimachia japonica* THUNB. were collected at Sugitani, Toyama, Japan, in June 1986 and a voucher specimen is on deposit at our institute.

Cell Lines The cell lines KB, L-5178Y, B-16, and PC-13 were supplied by the Department of Experimental Therapeutics, Cancer Research Institute, Kanazawa University, P-388 cell line was provided by the Research Laboratory, Toyama Chemical Co., Ltd. and HEp-2 cell lines were provided by the Department of Virology, School of Medicine, Toyama Medical and Pharmaceutical University. They were passaged in culture at our institute.

Cytotoxicity Assay *In vitro* cytotoxicity assays were carried out according to the standard National Cancer Institute guidelines.[5,6] The KB and HEp-2 cells were maintained on MEM media and B-16, PC-13, L-5178Y, and P-388 cells on RPMI 1640 media supplemented with 5% fetal calf serum. In the case of P-388, 2-mercaptoethanol (50 M) was also added to the medium. All assays were performed on tissue culture plates with 24 flat bottomed wells in a CO_2 incubator. After 72 h the KB, B-16, PC-13, and HEp-2 cells were determined according to the literature[7,8] and the number of viable cells was expressed as a percentages of that in a control incubation in which the cells were treated with the vehicle only. The L-5178Y and P-388 cells were also determined after 48 h incubation according to the literature.[9] The ED_{50} values were estimated from semi-log plots of the sample concentration (μg/ml) *vs.* the percent of viable cells.

Extraction and Separation The aerial parts of fresh *L. japonica* THUNB. (4.2 kg) were extracted with MeOH at room temperature for 3 d. The MeOH extract (190 g) was separated as shown in Fig. 1, and $CHCl_3$, H_2O, 95% MeOH, and petroleum benzin extracts were obtained (42, 145, 30, and 10 g, respectively). The 95% MeOH extract was chromatographed on silica gel by stepwise elution with $CHCl_3$, 5% MeOH/$CHCl_3$, 10% MeOH/$CHCl_3$, 20% MeOH/$CHCl_3$, 50% MeOH/$CHCl_3$, and MeOH. The fraction eluting with 5% MeOH/$CHCl_3$, which was most active against the KB cells among the eluted fractions, was rechromatographed on silica gel by stepwise elution with EtOAc/C_6H_6 mixtures to afford **1** (500 mg), **2** (1.5 g), **3** (52 mg), **4** (35 mg), **5** (100 mg), and **6** (300 mg).

Identification of Compounds 1—6 Compound **1** was obtained as colorless needles, mp 126—128 °C (EtOAc). Identification was established by comparisons of UV, IR, MS and ^{1}H-NMR data with published values[2] for 6-tridecylresorcylic acid (**1**). **2**, colorless needles, mp 83 °C (C_6H_6). Identification was established by comparisons of UV, IR, MS, and ^{1}H-NMR data with published values [2] for grevillol (**2**). **3**, yellow needles, mp above 310 °C, positive to $FeCl_3$ and reduction test for flavonoids. Acetylation of **3** afforded a tetraacetate, mp 185 °C. Identification was established by comparisons of the UV, IR, MS, and ^{1}H-NMR spectra with those of an authentic sample of kaempferol (**3**).**4**, yellow needles, mp above 310 °C, positive to $FeCl_3$ and reduction test for flavonoids. Acetylation of **4** afforded a pentaacetate, mp 198 °C. Identification was established by comparisons of the UV, IR, MS, and ^{1}H-NMR spectra with those of an authentic sample of quercetin (**4**). **5**, colorless plates, mp 139—141 °C (MeOH), positive to Liebermann–Burchard test for phytosterol. Compound **5** was identified as β-sitosterol (**5**) by direct comparison with an authentic sample. **6**, colorless micro crystals, mp 280—282 °C (MeOH/$CHCl_3$), positive to Liebermann–Burchard test for phytosterol. Compound **6** was identified as β-sitosterol-β-D-glucoside (**6**) by direct comparison with an authentic sample.

Preparation of 9—16 Preparations were in accordance with route 1 in Chart 1 and the procedures were as described.[3,4]

Preparation of Compounds **a**: A tetrahydrofuran (THF) solution (2 ml) of R-MgBr (prepared from the reaction of R-Br (2.5 mmol) and Mg (2.5 mmol) in THF under N_2, R = $CH_3(CH_2)_5$–, $CH_3(CH_2)_7$–, $CH_3(CH_2)_9$–, $CH_3(CH_2)_{13}$–, $CH_3(CH_2)_{15}$–, and $CH_3(CH_2)_{17}$–) was added little by little to 3 ml of THF solution of 5-dimethoxybenzoyl chloride (5 mmol) at −20 °C under N_2. The reaction mixture was allowed to stand until it reached room temperature and then separated in a usual manner to afford **a**. **19a** (yield, 41%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1600. MS *m/z*: 416 (M^+), 251, 165, 151. **20a** (yield, 94%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1600. MS *m/z*: 444 (M^+), 279, 165, 151. **21a** (yield, 45%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1600. MS *m/z*: 472 (M^+), 307, 165, 151. **22a** (yield, 68%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1710, 1600. MS *m/z*: 528 (M^+), 363, 165, 151. **23a** (yield, 62%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1600. MS *m/z*: 556 (M^+), 391, 165, 151. **24a** (yield, 93%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1715, 1600. MS *m/z*: 584 (M^+), 419, 165, 151.

Preparations of Compounds **b**: One of compounds **a** (0.5 mmol) was added to a 2 N KOH solution and the mixture was gently refluxed for 2 h, then extracted with EtOAc. The EtOAc extract was purified by silica gel column chromatography to afford the corresponding **b**. **19b** (yield, 82%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1600. MS *m/z*: 252 (M^+), 210, 168, 139. **20b** (yield, 80%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1600. MS *m/z*: 280 (M^+), 210, 168, 139. **21b** (yield, 23%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340, 1600. MS *m/z*: 308 (M^+), 210, 168, 139. **22b** (yield, 75%), colorless needles, mp 59—61 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340, 1600. MS *m/z*: 364 (M^+), 210, 168, 139. **23b** (yield, 69%), colorless needles, mp 47—49 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3360, 1600. MS *m/z*: 392 (M^+), 210, 168, 139. **24b** (yield, 83%), colorless needles, mp 45—46 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3340, 1600. MS *m/z*: 420 (M^+), 210, 168, 139.

Preparations of Compounds **c**: Et_3SiH (1.1 mmol) was added to a solution of one of compounds **b** (0.5 mmol) in CF_3COOH (4—15 eq) over 15 min, and the mixture was stirred at 45 °C for 24 h, then allowed to cool. Saturated $NaHCO_3$ solution (3 ml) was added, the mixture was extracted with EtOAc, and the extract was dried and evaporated. The residue was chromatographed on silica gel to afford the corresponding **c**. **19c** (yield, 44%), colorless, oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1600, 1460, 1175. MS *m/z*: 236 (M^+), 154, 153. **20c** (yield, 50%), colorless oil. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1600, 1460, 1175. MS *m/z*: 246 (M^+), 154, 153. **21c** (yield, 62%), colorless needles, mp 75—76 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1600, 1470, 1180. MS *m/z*: 292 (M^+), 154, 153. **22c** (yield, 53%), colorless needles, mp 48 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1600, 1465, 1170. MS *m/z*: 348 (M^+), 154, 153. **23c** (yield, 45%), colorless needles, mp 45 °C IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1600, 1460, 1175. MS *m/z*: 376 (M^+), 154, 153. **24c** (yield, 50%), colorless needles, mp 42 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1600, 1480, 1170. MS *m/z*: 404 (M^+), 154, 153.

Demethylation of Compounds **c**: A solution of one of compounds **c** (0.5 mmol) in dry CH_2Cl_2 was added at −20 °C to a solution of BBr_3 in dry CH_2Cl_2. The reaction mixture was protected from ingress of moisture by a drying tube containing $CaCl_2$, and allowed to warm up to room

temperature overnight. The mixture was then shaken with H_2O and extracted with EtOAc, and the extract was dried and evaporated. The residue was chromatographed on silica gel to afford 5-alkylresorcinol. **19** (yield, 32%), colorless needles, mp 56 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1600, 1475, 1150. ^{1}H-NMR (in CD_3OD) δ: 0.89 (3H, br t, J=6.8 Hz, $-CH_3$), 1.31 (8H, br s, $-CH_2-\times 4$), 1.56 (2H, br t, $-CH_2-$), 2.44 (2H, t, J=7.5 Hz, Ar–CH_2-), 6.08 (1H, t, J=2.2 Hz, Ar-H), 6.13 (2H, d, J=2.2 Hz, Ar-H×2). MS *m/z*: 222.159 (M^+, Calcd for $C_{14}H_{22}O_2$: 222.162). **20** (yield, 43%), colorless needles, mp 67 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1600, 1470, 1140. ^{1}H-NMR (in CD_3OD) δ: 0.89 (3H, br t, J=6.7 Hz, $-CH_3$), 1.28 (12H, br s, $-CH_2-\times 6$), 1.55 (2H, br t, $-CH_2-$), 2.43 (2H, t, J=7.5 Hz, Ar–CH_2-), 6.08 (1H, t, J=2.2 Hz, Ar-H), 6.12 (2H, d, J=2.2 Hz, Ar-H×2). MS *m/z*: 236.180 (M^+, Calcd for $C_{15}H_{24}O_2$: 236.178). **21** (yield, 38%), colorless needles, mp 79 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1600, 1470, 1140.^{1}H-NMR (in CD_3OD) δ: 0.89 (3H, t, J=6.8 Hz, $-CH_3$), 1.28 (14H, br s, $-CH_2-\times 7$), 1.56 (2H, br t, $-CH_2-$), 2.43 (2H, t, J=7.5 Hz, Ar–CH_2-), 6.08 (1H, t, J=2.2 Hz, Ar-H), 6.12 (2H, d, J=2.2 Hz, Ar-H×2). MS *m/z*: 264.211 (M^+, Calcd for $C_{17}H_{28}O_2$: 264.209). **22** (yield, 47%), colorless needles, mp 91—92 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3350, 1600, 1470, 1140. ^{1}H-NMR (in CD_3OD) δ: 0.89 (3H, t, J=6.8 Hz, $-CH_3$), 1.28 (24H, br s, $-CH_2-\times 12$), 1.56 (2H, br t, $-CH_2-$), 2.44 (2H, t, J=7.5 Hz, Ar–CH_2-), 6.10 (1H, t, J=2.1 Hz, Ar-H), 6.14 (2H, d, J=2.1 Hz, Ar-H×2). MS *m/z*: 320.267 (M^+, Calcd for $C_{21}H_{36}O_2$: 320.272). **23** (yield, 39%), colorless needles, mp 95 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1605, 1480, 1150. ^{1}H-NMR (in CD_3OD) δ: 0.91 (3H, t, J=6.6 Hz, $-CH_3$), 1.29 (28H, br s, $-CH_2-\times 14$), 1.57 (2H, br t, $-CH_2-$), 2.45 (2H, t, J=7.7 Hz, Ar–CH_2-), 6.10 (1H, t, J=2.2 Hz, Ar-H), 6.14 (2H, d, J=2.2 Hz, Ar-H×2). MS *m/z*: 348.306 (M^+, Calcd for $C_{23}H_{40}O_2$: 348.303). **24** (yield, 44%), colorless needls, mp 95—96 °C. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 1600, 1460, 1140. ^{1}H-NMR (in CD_3OD) δ: 0.89 (3H, t, J=6.4 Hz, $-CH_3$), 1.28 (32H, br s, $-CH_2-\times 16$), 1.56 (2H, br t, $-CH_2-$), 2.43 (2H, t, J=7.5 Hz, Ar–CH_2-), 6.09 (1H, t, J=2.2 Hz, Ar-H), 6.13 (2H, d, J=2.2 Hz, Ar-H×2). MS *m/z*: 376.339 (M^+, Calcd for $C_{25}H_{44}O_2$: 376.334).

Preparation of 30 A nitrobenzene solution of **22a** (0.15 mmol in 1.5 ml) and 12 ml of $(CH_3)_2SO$ were heated in an oil bath at 170 °C under a reflux condenser for 15 h, then cooled, and diluted with water. The mixture was extracted with petroleum benzin and the extract was purified by silica gel chromatography to gave **30** (yield, 71%) as a colorless amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2950, 2870, 1600, 1465, 1435, 1200, 1160, 1060, 930. MS *m/z*: 346 (M^+), 152.

Preparation of 32 PPC/Al_2O_3 (0.6 g, 0.6 mmol),[10] was added to a flask containing a solution of **22b** (0.12 mmol) in 3 ml of C_6H_6, and the mixture was stirred for 2 h. The solid filtered off, and washed with C_6H_6. The combined filtrate and washing were evaporated and purified by silica gel chromatography to gave **32** (yield, 92%) as colorless needles, mp 61—62 °C. MS *m/z*: 362 (M^+), 180, 165, 137. UV λ_{max}^{MeOH} nm (log ε): 227 (4.3), 261 (3.8), 314 (3.4). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2940, 2860, 1680, 1600, 1460, 1425, 1350, 1300, 1155, 1145, 1060.

Demethylation of 22b, 30, and 32 Demethylation was carried out as described for **c**. Demethylation of **22b** gave **29** (yield, 45%) as a colorless amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2950, 2870, 1600, 1460, 1340, 1160, 1150, 1000, 840. MS *m/z*: 336 (M^+), 318, 139, 124. ^{1}H-NMR (in CD_3OD) δ: 0.89 (3H, t, J=6.6 Hz, $-CH_3$), 1.27 (24H, br s, $-CH_2-\times 12$), 1.66 (2H, m, $-CH_2-$), 4.41 (1H, t, J=6.6 Hz, Ar–CH(OH)–), 6.15 (1H, t, J=2.2 Hz, Ar-H), 6.28 (2H, d, J=2.2 Hz, Ar-H×2). MS *m/z*: 336.262 (M^+, Calcd for $C_{21}H_{36}O_3$: 336.266). Demethylation of **30** gave **31** (yield, 73%) as a colorless amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2950, 2870, 1600, 1470, 1340, 1300, 1150, 1000, 970, 840. MS *m/z*: 318 (M^+), 134. ^{1}H-NMR (in CD_3OD) δ: 0.88 (3H, t, J=6.6 Hz, $-CH_3$), 1.26 (20H, br s, $-CH_2-\times 10$), 1.46 (2H, m, $-CH_2-$), 2.19 (2H, dt, J=6.8 Hz, $-CH_2-$), 6.21 (1H, dt, J=6.4, 15.6 Hz, =CH–), 6.31 (1H, d, J=15.6 Hz, Ar–CH=), 6.33 (1H, t, J=2.4 Hz, Ar-H), 6.50 (2H, d, J=2.4 Hz, Ar-H×2). MS *m/z*: 318.256 (M^+, Calcd for $C_{21}H_{34}O_2$: 318.256). Demethylation of **32** gave **33** (yield, 34%) as a colorless amorphous powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3250, 2920, 2850, 1660, 1600, 1470, 1340, 1170, 1155, 1005, 995. MS *m/z*: 334 (M^+), 152, 137. ^{1}H-NMR (in CD_3OD) δ: 0.89 (3H, t, J=6.6 Hz, $-CH_3$), 1.28 (22H, br s, $-CH_2-\times 11$), 1.68 (2H, m, $-CH_2-$), 2.90 (2H, t, J=7.3 Hz, Ar–CO–CH_2-), 6.48 (1H, t, J=2.4 Hz, Ar-H), 6.87 (2H, d, J=2.4 Hz, Ar-H×2). MS *m/z*: 334.248 (M^+, Calcd for $C_{21}H_{34}O_3$: 334.251).

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