# *In Vitro* Antitumor Activities of New Synthetic Bistetrahydrofuran Derivatives as Analogs of *Annonaceous* Acetogenins

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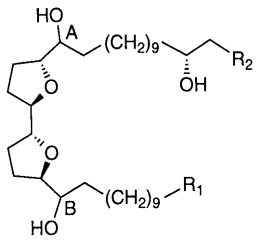
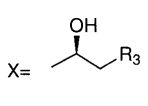
**We investigated the *in vitro* antitumor activities toward mouse and human cell lines of optically active synthetic bistetrahydrofuran (bis-THF) derivatives as analogs of *Annonaceous* acetogenins, which contain bis-THF, long unbranched alkyl chains, hydroxyl groups, and an α,β-unsaturated γ-lactone. These bis-THF derivatives were synthesized in a stereocontrolled manner, and have several modified structures at the alkyl side chains. We found that: 1) the unsaturated γ-lactone contributes to high potency in combination with the other less-functionalized alkyl chain, 2) the same absolute configuration of the bis-THF skeleton as that of the natural products produces more potent activity than the counterpart, 3) the alkyl chains and hydroxyl groups are crucial for exhibiting antitumor activity, 4) hydroxyl groups adjacent to the bis-THF skeleton may be replaced by amino or acylamino groups.**

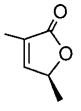
**Key words** *Annonaceous* acetogenins; bistetrahydrofuran; antitumor activity; structure–activity relationship

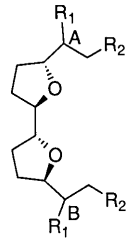
*Annonaceous* acetogenins are a new family of natural products containing diverse structures, and their miscellaneous biological activities have attracted much attention. In particular, because of their potent *in vitro* antitumor activities, the compounds with a neighboring bistetrahydrofuran (bis-THF) skeleton such as bullatacin (or squamocin G, **1**) and asimicin (or squamocin H, **2**), are considered to be potential lead compounds for further development of new therapeutic agents.[1–3] Selective toxicity to tumor cells was found in a preliminary *in vivo* investigation with mice.[2] Biochemical studies on the mode of action have revealed inhibitory action on mitochondrial respiration, and induction of apoptosis through a decrease in ATP production.[4] Their metal-binding affinities have also been investigated in relation to their biological action.[5] Natural products contain several types of structures, including mono-, bis-, or tri-THF, and much effort has been devoted to their total synthesis.[6] Structure–activity relationships of the natural products may be summarized as follows: 1) compounds with a neighboring bis-THF tend to be more potent than those with mono-THF, non-neighboring bis-THF, or tris-THF, 2) an α,β-unsaturated γ-lactone enhances the activity, 3) activity is dependent on the number and the position of the hydroxyl groups, 4) stereochemistry influences selectivity toward tumor cell lines, 5) the length of the alkyl chains has some influence on the activity. Because of the complexity in the structures of natural products, it seems to be difficult to clarify the role of each structural unit. Thus, we initiated a systematic investigation to define the role of each structural component in the *in vitro* antitumor activity, using simple synthetic compounds with a neighboring bis-THF moiety. Here we wish to describe in detail the synthesis and structure–antitumor activity relationships of these synthetic analogs (**3**—**22**), which differ in 1) the number of lactone moieties, 2) alkyl side




| (A, B) | $R_1$= | $R_2$= | $R_3$= |
|---|---|---|---|
| **1**: (*R*, *S*) | H | Lactone | - |
| **2**: (*R*, *R*) | H | Lactone | - |
| **3**: (*S*, *R*) | H | Lactone | - |
| **4**: (*R*, *S*) | H | $CH_2CO_2H$ | - |
| **5**: (*R*, *R*) | X | Lactone | Lactone |
| **6**: (*R*, *R*) | X | Lactone | $CH_2CO_2CH_3$ |
| **7**: (*R*, *R*) | X | $(CH_2)_2OH$ | $CH_2CO_2CH_3$ |
| **8**: (*R*, *R*) | X | $(CH_2)_2OH$ | $CH_2CH_3$ |




| (*A*, *B*) | $R_1$= | $R_2$= |
|---|---|---|
| **9**: (*R*, *R*) | OH | $(CH_2)_8CH_3$ |
| **10**: (*R*, *S*) | OH | $(CH_2)_8CH_3$ |
| **11**: (*S*, *S*) | OH | $(CH_2)_8CH_3$ |
| **12**: | =O | $(CH_2)_8CH_3$ |
| **13**: (*R*, *R*) | OH | $CH_3$ |
| **14**: (*R*, *S*) | OH | $CH_3$ |
| **15**: (*S*, *S*) | OH | $CH_3$ |
| **16**: (*R*, *R*) | OH | $(OCH_2CH_2)_2OEt$ |
| **17**: (*R*, *S*) | OH | $(OCH_2CH_2)_2OEt$ |
| **18**: (*R*, *R*) | NHAc | $(CH_2)_8CH_3$ |
| **19**: (*R*, *S*) | NHAc | $(CH_2)_8CH_3$ |
| **20**: (*S*, *S*) | NHAc | $(CH_2)_8CH_3$ |
| **21**: (*R*, *R*) | $NH_2$ | $(CH_2)_8CH_3$ |
| **22**: (*S*, *S*) | OH | OH |

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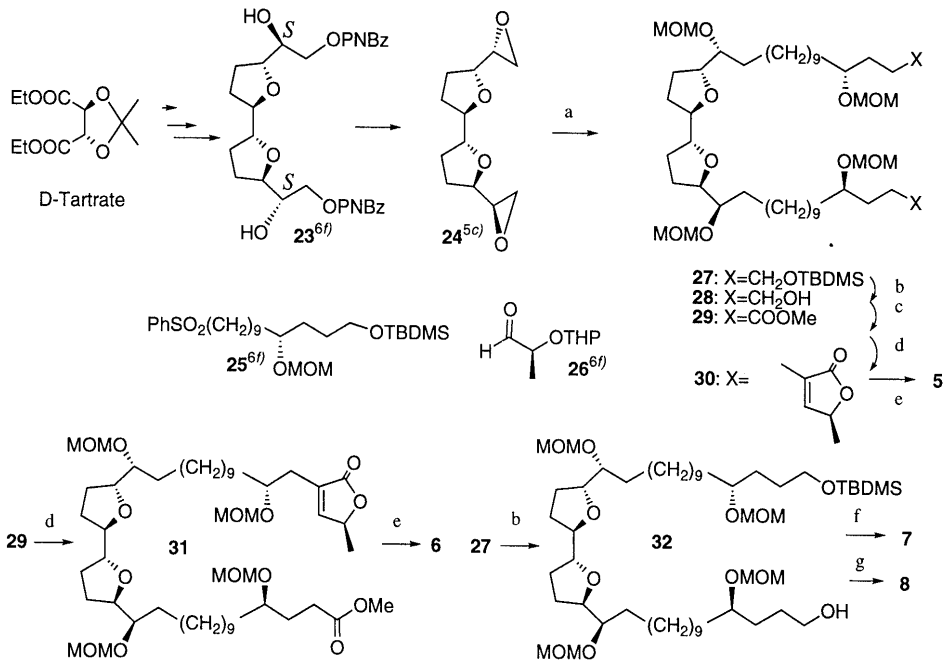


(a) 1) **25**, *n*-BuLi, THF, 2) MOMCl, *iso*-$Pr_2NEt$, $CH_2Cl_2$, 3) Na-Hg, EtOH, (b) *n*-$Bu_4NF$, THF, (c) 1) $CrO_3$-$H_2SO_4$, acetone, 2) $CH_2N_2$, $Et_2O$-AcOEt, (d) 1) LDA, THF, -78°C, 2) **26**, THF, -78°C, 3) 1 % CSA, MeOH-$H_2O$, 4) BzCl, pyridine, 0°C, 5) $NH_3$/MeOH, (e) $BF_3$•$Et_2O$, DMS, (f) 1) BzCl, pyridine, 2) $CrO_3$-$H_2SO_4$, acetone, 3) $CH_2N_2$, $Et_2O$-AcOEt, 0°C, 4) $K_2CO_3$, MeOH, 5) MOMCl, *iso*-$Pr_2NEt$, $CH_2Cl_2$, 6) $BF_3$•$Et_2O$, DMS, (g) 1) TsCl, pyridine, 2) $LiAlH_4$, THF, 3) *n*-$Bu_4NF$, THF, 4) $BF_3$•$Et_2O$, DMS.

Chart 1

chains, 3) stereochemistry, and 4) the functional groups attached next to the bis-THF skeleton.

**Synthesis** Aanalogs of the natural product with functionalized side chains were synthesized from the symmetric epoxide **24** by applying "*in situ* trapping" of the α-sulfonylcarbanion of **25**[6f] (Chart 1). The bis-THF intermediate **23**,[6f] which was synthesized from D-tartrate, was mesylated with methanesulfonyl chloride (MsCl), followed by hydrolysis and simultaneous epoxidation with *n*-$Bu_4NOH$ to produce **24**. A solution of *n*-BuLi was added to a mixture of **24** and **25** in dimethoxyethane (DME) to give the corresponding adduct, followed by desulfonylation with sodium amalgam (Na–Hg) to **27**, deprotection of the *tert*-butyldimethylsilyl groups (TBDMS) with *n*-$Bu_4NF$ to give **28**, oxidation with Jones reagent, and then esterification with $CH_2N_2$ to afford the ester **29**. Treatment of the ester **29** with lithium diisopropylamide (LDA), followed by the aldol addition with **26**, yielded the bis-aldol adduct as a major product, which gave the bislactone **30** *via* the following reactions: removal of the tetrahydropyranyl (THP) group and simultaneous lactonization with 10-camphorsulfonic acid (CSA), benzoylation with benzoyl chloride (BzCl), then elimination in $NH_3$–MeOH. Final deprotection of the methoxymethyl (MOM) groups of **30** in dimethylsulfide ($Me_2S$) in the presence of $BF_3 \cdot Et_2O$ produced **5**. The mono-aldol adduct was also obtained as a minor product in the aldol reaction between **29** and **26**, and was further transformed to the mono-lactone monoester derivative **6** through the precursor **31** by the same sequence of reactions as described for **5**. Partial deprotection of the symmetrical adduct **27** with *n*-$Bu_4NF$ gave the unsymmetric intermediate **32**, which was transformed to the unsymmetric analogues **7** *via* a sequence of reactions including benzoylation, deprotection of TBDMS and oxidation with Jones reagent, esterification with $CH_2N_2$, protection with MOMCl for ease of purification, then final deprotection of MOM groups in $Me_2S$ in the presence of $BF_3 \cdot Et_2O$. The derivative **8** having a reduced side chain was also obtained from **32** by means of the following reactions; tosylation with *p*-toluenesulfonyl chloride (TsCl), reduction with $LiAlH_4$, deprotection of TBDMS groups, then final deprotection of MOM groups. The monoacid **4**[6f] and the simple analogs **9**—**22**[5c] with less functionalized side chains were also synthesized from the intermediate **23**.

***In Vitro* Antitumor Activities** *In vitro* antitumor activities were measured using P388 mouse leukemia, PC-6 human lung cancer, and NUGC-3 human gastric cancer, and concentrations producing a 50% inhibitory effect ($GI_{50}$) are listed in Table 1. The natural products having the α,β-unsaturated γ-lactone (**1**, **2**) exhibited the most potent activities. The *bisepi*-isomer (**3**)[6f] retained high activity. The analog **4** with a carboxyl group instead of the lactone did not show potent activity. The additional lactone of **5** did not produce further enhancement of the activity. It is interesting that the model compounds with long alkyl chains (**9**, **10**, **11**) show antitumor activity, although the activities are low compared to those of the natural products. The analogs with functionalized side chains (**5**—**8**) showed only diminished activity similar to that of the simple model compounds. These results clearly indicate that the α,β-unsaturated γ-lactone contributes to high potency in combination with a less-functionalized

Table 1. *In Vitro* Antitumor Activities[a)]

| Compounds | Cancer cell line ($GI_{50}$ μg/ml) | | |
|---|---|---|---|
| | P388[b)] | PC-6[c)] | NUGC-3[d)] |
| **1** (Squamocin G) | 0.000104 | >0.250 | >0.250 |
| **2** (Squamocin H) | 0.000351 | >0.250 | >0.250 |
| **3** | 0.00223 | — | — |
| **4** | 0.577 | >1.250 | >1.250 |
| **5** | 0.0151 | >10.000 | 0.758 |
| **6** | 0.0917 | 1.788 | 1.860 |
| **7** | 0.680 | >2.500 | >2.500 |
| **8** | 0.0545 | 1.301 | 1.819 |
| **9** | 0.271 | 6.340 | — |
| enantio-**9** | 1.420 | 16.400 | — |
| **10** | 0.111 | 34.600 | — |
| enantio-**10** | 3.100 | >25.000 | — |
| **11** | 0.140 | 21.870 | — |
| **12** | >5.000 | — | — |
| **13** | >2.500 | >25.000 | >2.500 |
| enantio-**14** | 11.600 | >50.000 | — |
| **15** | >25.000 | >25.000 | >25.000 |
| **16** | >50.000 | >50.000 | >50.000 |
| **17** | 35.900 | >50.000 | >50.000 |
| **18** | 1.816 | >2.500 | >2.500 |
| **19** | 0.460 | >2.500 | >2.500 |
| **20** | >2.500 | >2.500 | >2.500 |
| **21** | 0.610 | 0.484 | 0.722 |
| **22** | 17.200 | 28.00 | — |

*a*) *In vitro* antitumor activities were assayed at the Exploratory Research Laboratories I, Daiichi Pharmaceutical Co., Ltd. *b*) P388 mouse leukemia. *c*) PC-6 human lung cancer. *d*) NUGC-3 human gastric cancer.

alkyl chain at the other side. The model compounds with the same absolute configuration of the bis-THF skeleton as the natural products are apparently more potent than their counterparts (**9** *vs.* enantio-**9**, and **10** *vs.* enantio-**10**), showing the importance of the stereochemistry. The fact that no activity was obtained with the ketone derivative **12** also indicates that the hydroxyl groups adjacent to the bis-THF play a critical role for antitumor activity. The model compounds with either short alkyl chains (**13**, **14**, **15**), ether chains (**16**, **17**) or without any alkyl chain (**22**) did not show activity, indicating that the alkyl chains are also essential for the activity. The hydroxyl groups adjacent to the THF moiety could be replaced by amino or acylamino groups (**18**—**21**). In particular, the amino derivative **21** exhibited potent activity toward human cell lines, suggesting that amino derivatives will be useful as a potential lead structure for development of new antitumor agents.

It has been found that the bis-THF compounds with long alkyl chains tend to form higher assemblies around metal cations probably due to van der Waals interaction.[5)] The present results also indicated that long alkyl chains have a significant role in the antitumor activities of the bis-THF analogs. These hydrophobic moieties may interact with a hydrophobic region of biomolecular targets for antitumor activity. On the other hand, as inhibition of mitochondrial respiration has been suggested as the major mode of action of *Annonaceous* acetogenins by McLaughlin and his co-workers,[4)] the long alkyl chains may be necessary for interaction with the plasma membrane, where the respiratory enzymes are located. The facts that antitumor activities were obtained with compounds having hydroxyl (**9**—**11**), amino (**18**, **19**), and acylamino (**21**) groups, but not with the ketone **12**, suggested that the functional groups adjacent to the bis-THF moiety act as a hydrogen-donor rather than an acceptor at a hydrogen bonding site of the target biomolecule. The next step should be identification of the molecular target of these natural products.

## Conclusion

We have synthesized a variety of compounds with the neighboring bis-THF moiety (**4**—**22**) for systematic investigation of the *in vitro* antitumor activity of *Annonaceous* acetogenins, and obtained the following results: 1) the lactone contributes to high potency in combination with the other less-functionalized alkyl chain, 2) the absolute configuration seen in the natural form affords a more potent activity than the counterpart, 3) the alkyl chains and hydroxyl groups are crucial for antitumor activity, 4) the hydroxyl groups adjacent to the bis-THF skeleton can be substituted by amino or amide functional groups. The amino bis-THF derivative may be advantageous compared to the natural products because of its improved solubility in water, and represents a potential lead structure for development of new antitumor agents.

## Experimental

$^1$H-NMR spectra were measured with a JEOL GX-270 spectrometer at 270 MHz. Chemical shifts are reported in ppm downfield from tetramethylsilane. IR spectra were taken on a JASCO IR Report-100 spectrometer and mass spectra were obtained with a JEOL JMS DX-300 or D-300 mass spectrometer. Optical rotations were measured on a JASCO DIP-370 polarimeter at room temperature.

**(4*R*,15*R*,16*R*,19*R*,20*R*,23*R*,24*R*,35*R*)-1,38-Bis(*tert*-butyldimethylsilyloxy)-16,19:20,23-diepoxy-4,15,24,35-tetrakis(methoxymethoxy)octatriacontane (27)** Under an argon atmosphere, *n*-BuLi (3.10 mmol) was added into a solution of **24**[5c)] (90 mg, 0.398 mmol) and the sulfone **25**[6f)] (820 mg, 1.59 mmol) in dry DME at room temperature. The reaction mixture was stirred for 20 min, quenched with saturated aqueous $NH_4Cl$, and diluted with ether. The organic layer was washed successively with water and brine, dried over $Na_2SO_4$, and evaporated. The residue was chromatographed (silica gel, AcOEt/hexane=2/3 to AcOEt) to give the sulfone adduct (440 mg, 88%) as a colorless oil. This sulfone (328 mg, 0.260 mmol) and iso-$Pr_2NEt$ (2.3 ml, 13.2 mmol) were dissolved in dry $CH_2Cl_2$ (8 ml), and MOMCl (0.6 ml, 7.90 mmol) was added at 0 °C under an argon atmosphere. The reaction mixture was stirred for 20 h at room temperature, diluted with ether, washed successively with saturated aqueous $NH_4Cl$, water and brine, dried over $Na_2SO_4$, then filtered and evaporated. The residue was chromatographed (silica gel, hexane/AcOEt=2/1 to 1/1) to give the tetrakis(methoxymethoxy) derivative (241 mg, 75%) as a colorless oil. A mixture of the above oil (378 mg, 0.281 mmol) and sodium amalgam (4.27 g) in absolute ethanol (8 ml) was stirred vigorously for 42 h at room temperature. The reaction mixture was quenched with aqueous $NH_4Cl$ and diluted with ether. The organic solution was successively washed with water and brine, dried over $Na_2SO_4$, then filtered and evaporated. The residue was chromatographed (silica gel, hexane/AcOEt=4/1 to AcOEt) to produce **27** (243 mg, 86%) as a colorless oil. $[\alpha]_D=+21.5°$ ($c=1.36$, $CHCl_3$), $^1$H-NMR ($CDCl_3$) $\delta$ 7.92—7.86 (4H, m), 7.68—7.52 (6H, m), 4.88—4.54 (4H, m), 4.64 (4H, s), 3.96—3.84 (4H, m), 3.63—3.54 (8H, m), 3.37—3.32 (12H, m), 3.26—3.24 (2H, m), 1.99—1.21 (52H, m), 0.89 (18H, s), 0.04 (12H, s); IR (neat) $cm^{-1}$: 1255, 1100, 1040, FAB-MS *m/z*: 1085 $(M+Na)^+$, HR-FAB-MS Calcd for $C_{58}H_{118}O_{12}Si_2Na$ 1085.8060, Found 1085.8068.

**(2*R*,13*R*,14*R*,17*R*,18*R*,21*R*,22*R*,33*R*)-14,17:18,21-Diepoxy-2,13,22,-33-tetrahydroxy-1,34-bis[(4*S*)-4-methyl-γ-butyrolactone-2-en-2-yl]tetratriacontane (5)** A mixture of **28** (42 mg, 0.0395 mmol) and *n*-$Bu_4NF$ (0.24 mmol) in dry THF (1.5 ml) was stirred for 2 h at room temperature.

The reaction mixture was diluted with ether, washed successively with saturated aqueous $NH_4Cl$, water and brine, dried over $Na_2SO_4$, filtered and evaporated. The residue was chromatographed (silica gel, hexane/AcOEt = 1/1 to AcOEt/EtOH = 9/1) to give the diol **28** (31 mg, 94%) as a colorless oil. Jones reagent (8 M, $CrO_3$–$H_2SO_4$, 0.2 ml) was added to a solution of **29** (15 mg, 0.018 mmol) in acetone (1 ml) at −20 °C, and the whole was stirred for 5 min, followed by further addition of Jones reagent (8 M, $CrO_3$–$H_2SO_4$, 0.1 ml). The reaction mixture was quenched with saturated aqueous $NaHCO_3$, and diluted with AcOEt. The organic solution was washed successively with water and brine, then dried over $Na_2SO_4$ and evaporated. The crude product was treated with $CH_2N_2$ in EtOAc/ether, then quenched with AcOH. Filtration and evaporation gave a residue, which was chromatographed (silica gel, hexane/AcOEt = 2/1 to 1/1) to give the ester **29** (10 mg, 63%) as a colorless oil. Under an argon atmosphere, a solution of **29** (23 mg, 0.026 mmol) in dry THF (0.1 ml) was added to a solution of LDA (1.63 M in dry THF 80 $\mu$l) at −78 °C for 10 min, and the mixture was stirred for 20 min at the same temperature. A solution of **26**[6f] (18 mg, 0.105 mmol) in dry THF (100 $\mu$l) was added to it, and the whole was stirred for 10 min at the same temperature. It was quenched with saturated aqueous $NH_4Cl$, warmed up to room temperature, then extracted with ether. The organic layer was separated and washed successively with water and brine, dried over $Na_2SO_4$, then filtered and evaporated. The residue was chromatographed (silica gel, hexane/AcOEt = 4/1) to give the bis aldol adduct (26 mg) together with the mono adduct (2 mg, 2.19 $\mu$mol, 8%). A mixture of the above bis-aldol adduct and CSA (1% MeOH/$H_2O$ = 9/1) was stirred for 1 h at room temperature. The reaction mixture was quenched with saturated aqueous $NaHCO_3$, and diluted with ether. The organic solution was washed successively with water and brine, dried over $Na_2SO_4$, filtered and evaporated. The residue was dissolved in dry pyridine (0.6 ml), followed by the addition of benzoyl chloride (0.2 ml, 1.72 mmol). This mixture was stirred for 2 h at room temperature, diluted with ether, washed successively with aqueous saturated $NaHCO_3$, water and brine, dried over $Na_2SO_4$, then filtered and evaporated. The residue was dissolved in 21% $NH_3$/MeOH (2.5 ml) and stirred for 1.5 h at room temperature. The mixture was diluted with ether, washed successively with aqueous saturated $NaHCO_3$, water and brine, dried over $Na_2SO_4$, filtered and evaporated. The residue was chromatographed (silica gel, hexane/AcOEt = 4/1 to 1/1) to give **30** (3 $\mu$g, 13% from **29**) as a colorless oil. A solution of **30** (1.2 mg, 1.28 mmol) and $BF_3 \cdot Et_2O$ (15 $\mu$l) in $Me_2S$ (0.12 ml) was stirred for 1.5 h at room temperature, and the mixture was quenched with aqueous saturated $NaHCO_3$. The reaction mixture was diluted with AcOEt and the organic layer was washed successively with water and brine, dried over $Na_2SO_4$, then filtered and evaporated. The residue was chromatographed (silica gel, AcOEt) followed by purification by HPLC (nacalai tesque COSMOSIL 5C18-MS, MeOH/$H_2O$ = 17/3) to give the bislactone **5** (0.6 mg, 63%) as colorless crystals. mp 52—55 °C; $^1$H-NMR ($CDCl_3$) $\delta$ 7.19 (2H, d, $J$ = 1.4 Hz), 5.06 (2H, ddd, $J$ = 6.9, 6.9, 5.5 Hz), 3.89—3.82 (6H, m), 3.40—3.39 (2H, m), 2.55—2.51 (2H, m), 2.42—2.39 (2H, m), 2.04—1.12 (48H, m), 1.44 (6H, d, $J$ = 6.9 Hz); FAB-MS $m/z$: 763 $(M+H)^+$, HR-FAB-MS Calcd for $C_{44}H_{75}O_{10}$ 763.5360, Found 763.5342.

**(+)-(4*R*,15*R*,16*R*,19*R*,20*R*,23*R*,24*R*,35*R*)-16,19:20,23-Diepoxy-4,15,24,35-tetrahydroxy-36-[(4*S*)-4-methyl-γ-butyrolactone-2-en-2-yl]-hexatriacontanoic Acid Methylester (6)** The mono aldol adduct (2 mg, 2.19 $\mu$mol) obtained in the synthesis of **30** was converted to **6** (0.4 mg, 25%) as colorless crystals through the tetrakis(methoxymethoxy) derivative **31** by a similar procedure to that described above. $^1$H-NMR ($CDCl_3$) $\delta$ 7.19 (1H, d, $J$ = 1.3 Hz), 5.05—5.00 (1H, m), 4.04—3.89 (4H, m), 3.86—3.80 (1H, m), 3.67 (3H, s), 3.66—3.50 (1H, m), 3.50—3.44 (2H, m), 2.50 (2H, m), 2.41 (2H, m), 1.95—1.25 (50H, m), 1.44 (3H, d, $J$ = 6.9 Hz); FAB-MS $m/z$: 761 $(M+Na)^+$, HR-FAB-MS Calcd for $C_{42}H_{74}O_{10}Na$ 761.5180, Found 761.5198.

**(+)-(4*R*,15*R*,16*R*,19*R*,20*R*,23*R*,24*R*,35*R*)-16,19:20,23-Diepoxy-4,15,24,35,38-pentahydroxyoctatriacontanoic Acid Methylester (7)** A solution of **27** (42 mg, 0.040 mmol) and $n$-$Bu_4NF$ (0.039 mmol) in THF (1 ml) was stirred for 40 min at room temperature followed by the addition of $n$-$Bu_4NF$ (0.039 mmol). After having been stirred for 2 h, the mixture was diluted with ether, washed successively with saturated aqueous $NH_4Cl$, water and brine, dried over $Na_2SO_4$, then filtered and evaporated. The residue was chromatographed (silica gel, hexane/AcOEt = 4/1 to AcOEt/EtOH = 9/1) to give the monosilylated compound **32** (18 mg, 48%) as a colorless oil together with the diol **28** (16 mg, 48%). A solution of **32** (5 mg, 5.27 mmol) and benzoyl chloride (10 $\mu$l, 0.086 mmol) in dry pyridine (0.5 ml) was stirred for 2 h at room temperature under an argon atmosphere. The reaction mixture was diluted with ether, washed successively with aqueous saturated $NaHCO_3$, water and brine, dried over $Na_2SO_4$, filtered and evaporated. The residue was chromatographed (silica gel, hexane/AcOEt = 4/1 to AcOEt/EtOH = 9/1) to give the benzoate (5 mg, 91%) as a colorless oil. After desilylation, oxidation, and esterification by similar procedures to those described for **29**, the benzoate (4 mg, 3.8 mmol) gave the protected form of **7** (3 mg, 81%) as a colorless oil. This product (5 mg, 6.14 $\mu$mol) was debenzoylated in dry MeOH (0.5 ml) in the presence of a trace amount of $K_2CO_3$ followed by deprotection *via* a similar procedure to that described for **5** to afford **7** (1.4 mg, 33%) as colorless crystals. $^1$H-NMR ($CDCl_3$) $\delta$ 3.89—3.82 (2H, m), 3.72—3.60 (3H, m), 3.68 (3H, s), 3.40—3.38 (2H, m), 2.47 (2H, t, $J$ = 6.9 Hz), 2.00—1.96 (2H, m), 1.95—1.26 (60H, m); FAB-MS $m/z$: 687 $(M+H)^+$, 709 $(M+Na)^+$; HR-FAB-MS Calcd for $C_{39}H_{75}O_9$ 687.5411, Found 687.5425.

**(+)-(4*R*,15*R*,16*R*,19*R*,20*R*,23*R*,24*R*,35*R*)-16,19:20,23-Diepoxy-1,4,15,24,35-pentahydroxyoctatriacontane (8)** *p*-TsCl (8 mg, 0.042 mmol) was added to a solution of **32** (13 mg, 0.014 mmol) in dry pyridine (0.1 ml) at −10 °C under an argon atmosphere, and the mixture was stirred for 18 h in an ice bath. It was then diluted with AcOEt, washed successively with saturated aqueous $NaHCO_3$, water and brine, dried over $Na_2SO_4$, filtered and evaporated. The residue was chromatographed (silica gel, hexane/AcOEt = 4/1 to AcOEt) to give the corresponding tosylate (11 mg, 72%) as a colorless oil. A solution of the above oil (10 mg, 9.1 $\mu$mol) and $LiAlH_4$ (8 mg, 0.21 mmol) in dry THF was refluxed for 2 h. The reaction mixture was worked up according to the general procedure to give a crude oil, which was chromatographed on a silica gel column to give the reduced product (4.2 mg, 50%) as a colorless oil. The protective groups were removed as described above, and chromatographed (silica gel, hexane/AcOEt = 1/1 to AcOEt, then MeOH) to give **8** (1 mg, 39%) as colorless crystals. $^1$H-NMR ($CDCl_3$) $\delta$ 3.89—3.82 (2H, m), 3.70—3.62 (3H, m), 3.40—3.38 (2H, m), 2.48—2.46 (1H, m), 2.00—1.95 (2H, m), 1.95—1.26 (61H, m), 0.93 (3H, t, $J$ = 6.3 Hz); FAB-MS $m/z$: 643 $(M+H)^+$, 665 $(M+Na)^+$; HR-FAB-MS Calcd for $C_{38}H_{75}O_7$ 643.5512, Found 643.5522.

**Bioassays** Cytotoxicity to P388 mouse leukemia, PC-6 human lung cancer, and NUGC-3 human gastric cancer (Table 1) was evaluated at the Exploratory Research Laboratories I, Daiichi Pharmaceutical Co., Ltd., Japan, using modifications of the standard protocols of the National Cancer Institute.[7]

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