Synthesis and Antitumor Activity of New Amphiphilic Alkylglycerolipids Substituted with a Polar Head Group, 2-(2-Trimethylammonioethoxy)ethyl or a Congeneric Oligo(ethyleneoxy)ethyl Group

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A new series of amphiphilic 1-octadecyl glycerolipids (eleven compounds, 1a—k) were designed and synthesized, in which the 3-phosphocholine portion of platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF) was replaced by the 2-(2-trimethylammonioethoxy)ethyl group and congeneric groups having oligo(ethyleneoxy)ethyl bridges of various lengths at position 3, together with modification at position 2 (lower alkyl, acetonyl, acetoacetyl, carboxymethyl and pyrimidin-2-yl groups). These ether lipids, characterized by a nonphosphorus lysoglycerolipid structure, showed potent antitumor activity *in vitro* (human promyelocytic leukemia cells, HL-60, and human epidermoid carcinoma cells, KB) and *in vivo* (mouse sarcoma S180 and mouse mammary carcinoma MM46). Maximal *in vitro* potency was obtained with 1-*O*-octadecyl-2-*O*-(2-pyrimidinyl)-3-*O*-[2-(2-trimethylammonioethoxy)ethyl]glycerol (1g; IC_{50} values for both HL-60 and KB were 0.32 μg/ml, indicating a higher activity than alkyl-lysophospholipid, ET18-OMe). Several appropriately 2-substituted 1-octadecylglycerolipids with the 3-[2-(2-trimethylammonioethoxy)ethyl] group (*e.g.*, methyl, 1b; butyl, 1f; 2,2,2-trifluoroethyl, 1j; and acetonyl, 1k) showed a potent life-span-prolonging effect on mice with ascites sarcoma S180 and on those with mammary carcinoma MM46, when administered intraperitoneally at 16.5 and 12.5 mg/kg/d, respectively. Compounds 1b and 1k showed definite tumor growth inhibition against solid sarcoma S180 in mice, whether given *p.o.* or i.v. at 16.5 mg/kg/d. Studies on the structure–activity relationships indicate that the metabolic stability to phospholipase C or related enzymes is at least partly responsible for the potent antitumor activity of this series of ether lipids.

Keywords alkyl glycerolipid; alkyl lysophospholipid; 1-*O*-octadecyl-2-*O*-(2-pyrimidinyl)-3-*O*-[2-(2-trimethylammonioethoxy)ethyl]glycerol; nonphosphorus-lysoglycerolipid; antitumor activity; sarcoma S180; mammary carcinoma MM46; human promyelocytic leukemia cell HL-60; human epidermoid carcinoma cell KB; structure–activity relationship

As a new approach to cancer chemotherapy, much attention has been focused on bioactive alkyl ether phospholipids (analogs of platelet-activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF)) because of their direct antitumor effects and effects on the host defense system.[2)] A typical agent in this class is 1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine (ET 18-OMe).[3)] However, its use in therapy has been limited by undesirable side effects including PAF agonistic action.[4)] More recently, ilmofosine (BM-41440)[5)] and hexadecylphosphocholine (HPC)[6)] have been reported and both are currently under clinical study.

In an earlier report,[7)] we described the synthesis and antitumor activity of 1-*O*-octadecyl-2-*O*-acetoacetylglycerophospholipids having an extended methylene bridge in the 3-polar head side chain. These phospholipids exhibited highly potent activity against ascites sarcoma S180 (S180) and mammary carcinoma MM46 in mice. However, further evaluation revealed that the compounds with the ω-(trimethylammonio)decylphosphate group at position 3 only moderately inhibited the growth of solid S180 in mice in contrast to their potent life-prolonging effect on mice bearing ascites S180. The poor effect on the solid tumor can be explained by assuming that this compound is metabolically less stable and thus cannot be delivered to the target tumor cells. The 2-acyl ester and 3-phosphodiester bonds of this compound are probably susceptible to attack by phospholipases A_2 (PLase A_2) and phospholipase C and/or D (PLase C and/or D), respectively, which exist in various organ tissues in mammals.[8)] In direct contrast to this hypothesis is that proposed by German researchers,[6,9,10)] that ether phospholipids are needed as a substrate for PLase C or related enzymes in order for them to exert their antitumor activity, because the cytotoxic principles are not the ether lipids themselves but their metabolites formed by the action of these enzymes, presumably the corresponding apolar alcohols.

These two hypotheses are not compatible and the discrepancy raises some interesting questions. To answer them, we decided to synthesize an ether lipid which differed from previously known alkyl-lysophospholipids by the lack of a phosphoryl group and by the replacement of the phosphocholine group with an ether-linked choline or related group as a polar-head side chain at position 3 of the glycerol moiety. Another reason for our interest is the fact that an ether lipid of this type is cationic and can be presumed to have a much higher affinity for malignant cells than negatively charged or zwitter ionic ether lipids, including alkyl-lysophospholipids, due to Coulomb's force, because the cell surface of malignant cells has been proved to be much more electronegatively charged than that of normal cells.[11,12)]

We thought it to be of particular interest to synthesize and evaluate cationic alkyl-ether lipids which could sufficiently resist the action of these PLases to reach and bind to the sites, probably the surface membrane of the tumor cells, where they could exert their inhibitory activity.

In a continuation of our studies[7,13–18)] on antitumor ether lipids, we describe here the synthesis and antitumor activity of new amphiphilic alkylglycerolipids, in which primarily the 3-phosphocholine moiety of the PAF molecule was replaced by a 2-(2-trimethylammonioethoxy)ethyl or a congeneric oligo(ethyleneoxy)ethyl group.

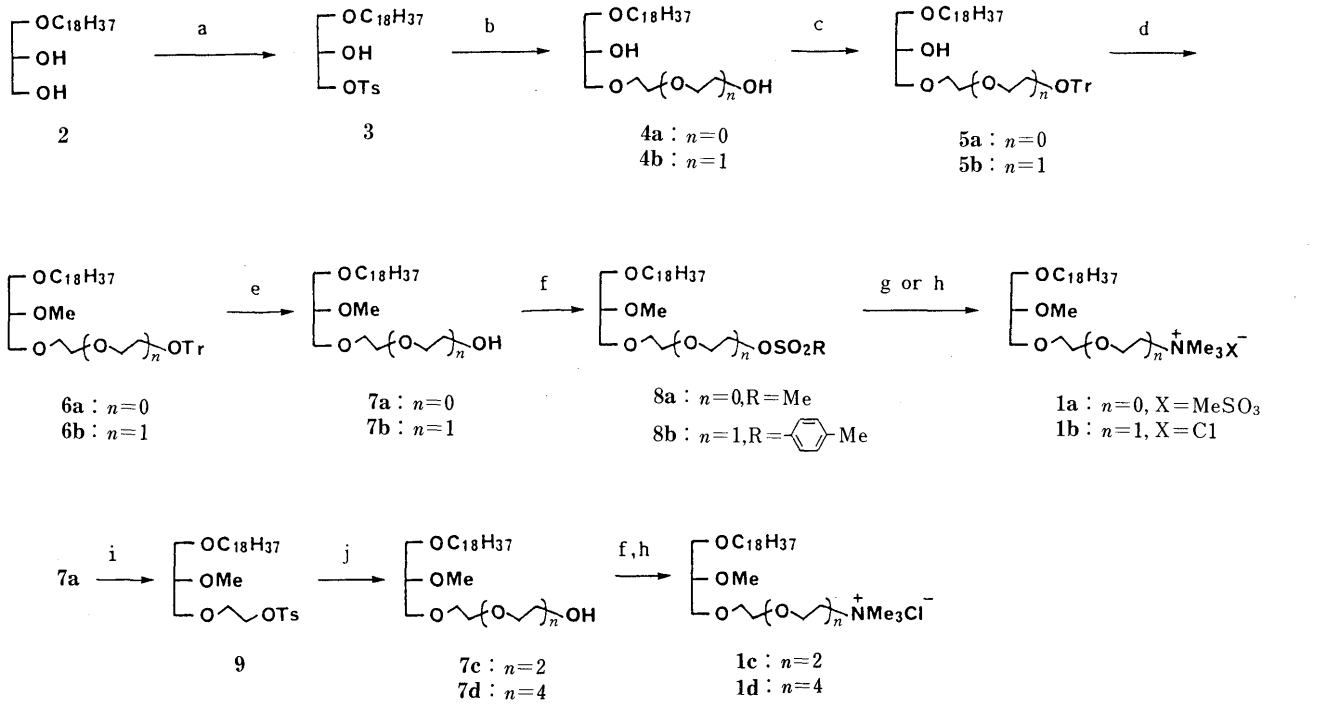
Chemistry The structural features of the ether glycero-

lipids studied are: (a) an ether-linked alkyl moiety at position 1, (b) a nonmetabolizable small substituent at position 2, and (c) an ether-linked polar-head side chain at position 3, *i.e.*, a 2-(2-trimethylammonioethoxy)ethyl or a congeneric oligo(ethyleneoxy)ethyl group. Although compounds **1a**—**k** are characterized by the absence of a phosphoryl group in the 3-polar head side chain, they are amphiphilic, as are the previously known antitumor alkyllysophospholipids. These unnatural ether lysolipids were synthesized in two ways which involved 1) the use of the intermediary 2-substituted 1-octadecylglycer-3-yl ether of mono- and oligoethylene glycol (**7a**—**d**, **j** and **k**; Charts 1 and 3) or 2) the use of the lysolipid (**12**, Chart 2) as a key intermediate.

Tosylation of the primary hydroxyl group of 1-*O*-octadecylglycerol (**2**) and subsequent etheration with ethyleneglycol and diethyleneglycol in the presence of NaH gave the 3-(2-hydroxyethyl)- and the 3-[2-(2-hydroxyethoxy)ethyl]ethers of **2** (**4a**, **b**), respectively. The desired monoglyceryl derivatives (**4a**, **b**) of ethylene glycol and diethylene glycol were easily isolated from the corresponding α,ω-diglyceryl ethers of mono- and diethylene glycols as minor products by column chromatography. Tritylation of the 3-ω-primary hydroxyl group of **4a** and **4b** and the following methylation of the 2-*sec*-hydroxyl group of the 1,3-disubstituted glycerols (**5a**, **b**) with methyl *p*-toluenesulfonate yielded 2-*O*-methyl-1-*O*-octadecyl-3-(2-trityloxyethyl)glycerol (**6a**) and the corresponding 3-[2-(2-trityloxyethoxy)ethyl]ethers (**6b**), respectively. Acid hydrolysis of **6a** and **6b** gave the corresponding 3-(2-hydroxyethyl)- and the 3-[2-(2-hydroxyethoxy)ethyl]ether (**7a**, **b**), respectively. These hydroxy ethers (**7a**, **b**) were converted to the mesylate (**8a**) or tosylate (**8b**) which, on treatment with trimethylamine, gave the desired trimethylammonium compounds (**1a**, **b**).

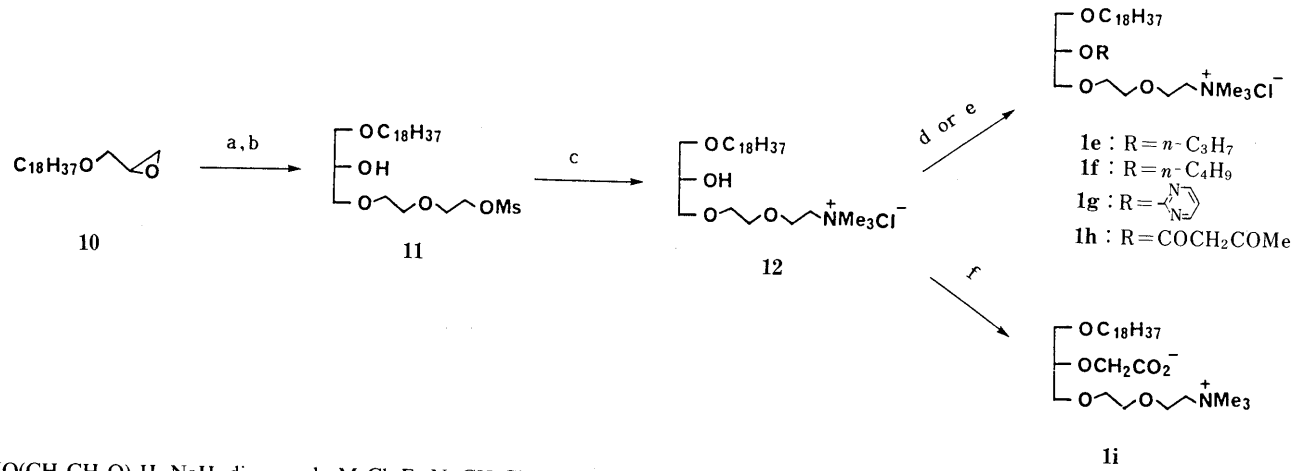
1-*O*-Octadecyl-2-*O*-methylglycerol with an oligo(ethyleneoxy)ethyl unit [*i.e.*, **1c**, $n=2$, the 3-(ω-trimethylammonio-3,6-dioxaoctyl)ether, and **1d**, $n=4$, the 3-(ω-trimethylammonio-3,6,9,12-tetraoxatetradecyl)ether] were synthesized, respectively, from the mono(1-octadecylglyceryl)-ethers of triethylene glycol (**7c**) and pentaethylene glycol (**7d**) by tosylation (or mesylation) of the ω-hydroxy group, followed by the reaction with trimethylamine in a similar manner to that mentioned above. The intermediates **7c** and **7d** were prepared by the coupling reaction of 1,2-di-*O*-substituted 3-(2-tosyloxyethyl)glycerol (**9**) with diethylene glycol and tetraethylene glycol, respectively, in alkali. In methylation of the 2-hydroxy group of 1,3-di-*O*-substituted glycerol (**5a**, **b**) and in the conversion reaction of the sulfonate (**9** and **13**) to the corresponding ethers (**7c**, **d** and **6j**), the use of a phase-transfer catalyst (*e.g.*, cetyltrimethylammonium chloride) was essential for the smooth progress of the reaction.[19]

As described later, the antitumor activity of the 3-[2-(2-trimethylammonioethoxy)ethyl]ether (**1b**) was the most potent among this series of 2-methyl-1-octadecylglycerolysolipids (**1a**—**d**). This led us to synthesize further analogs of 3-[2-(2-trimethylammonioethoxy)ethyl]ethers having an appropriate substituent at position 2 as a structural feature (**1e**—**k**). The synthetic routes shown in Chart 2 were selected because of the ready availability of the key intermediate (**12**) for synthesizing the desired 2-substituted analogs (**1e**—**i**). 1-Octadecyl glycidyl ether (**10**)[20] was used as a starting compound. Treatment of **10** with diethyleneglycol in the presence of NaH gave 3-*O*-[2-(2-hydroxyethoxy)ethyl]-1-*O*-octadecylglycerol (**4b**) which, on



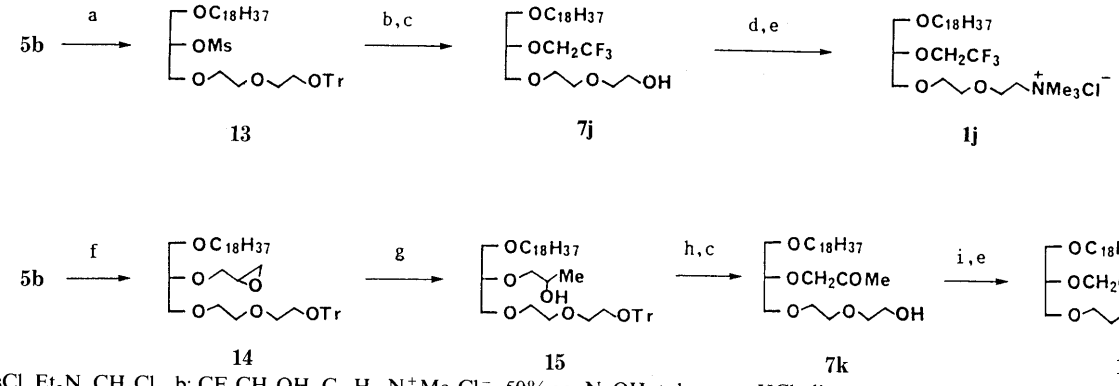
a: TsCl, pyridine, CH_2Cl_2. b: $HO(CH_2CH_2O)_nH$ ($n=1$ or 2), NaH, dioxane. c: trityl chloride, pyridine. d: TsOMe, $C_{16}H_{33}N^+Me_3Cl^-$, 50% aq. NaOH, toluene. e: HCl, dioxane, MeOH. f: MsCl (or TsCl), Et_3N, CH_2Cl_2. g: 20% Me_3N–toluene. h: 30% aq. Me_3N, THF; Amberlite IRA-410 [Cl], MeOH. i: TsCl, Et_3N, toluene. j: $HO(CH_2CH_2O)_nH$ ($n=2$ or 4), $C_{16}H_{33}N^+Me_3Cl^-$, 50% aq. NaOH, dioxane.

Chart 1



a: $HO(CH_2CH_2O)_2H$, NaH, dioxane. b: MsCl, Et_3N, CH_2Cl_2. c: 30% aq. Me_3N, EtOH, THF; sat. aq. NaCl, CH_2Cl_2. d: n-C_3H_7I (or n-C_4H_9I or 2-chloropyrimidine), 50% aq. NaOH, CH_2Cl_2; Amberlite IRA-410 [Cl], MeOH. e: diketene, pyridine. f: $ClCH_2COOH$, 50% aq. NaOH, dioxane; Amberlite CG-50 [H], THF–H_2O (9 : 1).

Chart 2



a: MsCl, Et_3N, CH_2Cl_2. b: CF_3CH_2OH, $C_{16}H_{33}N^+Me_3Cl^-$, 50% aq. NaOH, toluene. c: HCl, dioxane, MeOH. d: MsCl, Et_3N, toluene, hexane. e: 30% aq.Me_3N, THF, EtOH; Amberlite IRA-410 [Cl], MeOH. f: epibromohydrin, NaH, hexane. g: $LiAlH_4$, Et_2O. h: CrO_3–H_2SO_4, acetone. i: TsCl, Et_3N, THF.

Chart 3

mesylation, followed by reaction with trimethylamine, gave a lysolipid **12** {(1-*O*-octadecyl-3-[2-(2-trimethylammonioethoxy)ethyl]glycerol}. Etheration of the 2-hydroxyl group of **12** by treatment with *n*-propyl iodide, *n*-butyl iodide, 2-chloropyrimidine, and chloroacetic acid in the presence of 50% sodium hydroxide in an organic solvent gave the desired 2-substituted glyceryl ethers (**1e**—**g**, and **i**), respectively. In this reaction, no phase transfer catalyst was required, because the amphiphilic lysolipid **12** probably served as a catalyst. For synthesis of the 2-acetoacetyl ester (**1h**), diketene was used as an acylating agent in the reaction with compound **12**.

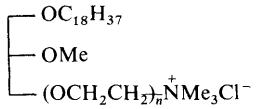
For the synthesis of 2-(2,2,2-trifluoroethyl)- and 2-acetonyl ethers (**1j**, **k**), direct etheration of the lysolipid **12** was not suitable, probably due to the low reactivity of both 2,2,2-trifluoroethyl iodide and 1-bromo-2,2-dimethoxypropane, and the insufficient stability of compound **12** in alkali. Thus, the alternative route using an intermediary 1,3-di-*O*-substituted glycerol (**5b**) was employed for synthesis of these analogs. Compounds **1j** and **1k** were prepared by five- and six-step reactions, respectively, starting from **5b** as shown in Chart 3. Compound **6j**, the key intermediate for the synthesis of **1j**, was obtained by the reaction of the mesylate (**13**) with 2,2,2-trifluoroethanol in the presence of phase transfer catalyst. On treatment with epibromohydrin, **5b** provided the 1,3-di-*O*-substituted 2-*O*-(2,3-epoxypropyl)glycerol (**14**), which on reduction with $LiAlH_4$, followed by Jones oxidation, gave the corresponding 2-acetonyl ether (**6k**). The intermediates **6j** and **6k** were converted smoothly to the desired products (**1j** and **1k**) by hydrolysis, mesylation or tosylation and finally amination (Chart 3).

The present ether glycerolipids (**1a**—**k**) have a chiral carbon in the glycerol moiety at position 2 and this chirality is presumed to have an influence upon the antitumor activity. However, all of the compounds listed in Tables I and II are racemic. They were purified by column chromatography and, in some cases, by crystallization from a suitable solvent system. The structure and purity of the products were confirmed by thin-layer chromatography, nuclear magnetic resonance (NMR) spectroscopy and elemental analysis. They were amphiphilic and showed good solubility both in water and in a variety of organic solvents, which is advantageous for biological studies.

Biological Results and Discussion

In order to obtain an alkyl ether lipid with potent antitumor activity and reduced undesirable side effects, we

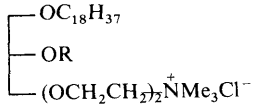
TABLE I. Antitumor Activity



	1a $n=1$	1b 2	1c 3	1d 5	ET18-OMe
In vitro, IC_{50} (μg/ml)[a]					
HL-60	1.25	1.25	0.62	1.25	2.5
KB	0.16	0.32	0.16	0.16	0.63
In vivo, *T/C* (%)[a]					
S180	241[e] (1/5)[b]	394[c] (0/5)	247[d] (0/5)	169[e] (0/3)	235[e] (0/5)
MM46	—	(5/5)	117 (2/5)	139 (2/5)	214[e] (3/5)

a) Procedures, see the corresponding sections in Experimental. *b*) No. of long-term survivors/No. of treated mice. The survivors on day 60 given in parenthesis (the numerator) in this table were not included in the calculation of *T/C* (%). *c*) $p<0.001$. *d*) $p<0.01$. *e*) $p<0.05$.

TABLE II. Antitumor Activity



	1b R=–Me	1e –C_3H_7	1f –C_4H_9	1g 	1h –$COCH_2COCH_3$	1i –$CH_2CO_2^-$	1j –CH_2CF_3	1k –CH_2COMe	ET18-OMe
In vitro, IC_{50} (μg/ml)									
HL-60	1.25	1.25	2.5	0.31	20	2.5	2.5	1.25	2.5
KB	0.32	—	—	0.32	—	0.32	—	0.32	0.63
In vivo									
Inhibition ratio (%)									
S180 s.c.–i.v.	65	18	—	41	36.5	—	44	78	50
s.c.–*p.o.*	77	53	75	40	—	—	73[a]	77	50
T/C (%) i.p.–i.p.									
S180	394[c] (0/5)	277[d] (1/5)	220[c] (3/5)[b]	200[d] (0/5)	292 (2/5)	173[d] (0/5)	268[c] (1/5)	220[d] (3/5)	235[d] (0/5)
MM46	(5/5)	154 (4/5)	—	160 (4/5)	126[d] (3/5)	125 (4/5)	(5/5)	162[d] (2/5)	214[e] (3/5)
P388	145[d] (0/5)	169[e] (0/5)	137[d] (0/5)	129[e] (0/5)	106 (0/5)	111 (0/5)	—	102 (0/5)	108 (0/5)

a) Dose: 1 mg/mouse. *b*) Dose: 0.17 mg/mouse. *c*) $p<0.001$. *d*) $p<0.01$. *e*) $p<0.05$.

designed and synthesized a new series of amphiphilic glycerol triethers which were expected to be resistant to the action of PLase (A_1, A_2, C and D) and thus could be expected to reach, become incorporated into and accumulate in the surface membrane of malignant cells. To examine the antitumor activity of the eleven new compounds, we used human promyelocytic leukemia cells (HL-60) and epidermoid carcinoma cells (KB) in *in vitro* tests, and the mouse sarcoma S180 and mammary carcinoma MM46 in *in vivo* tests. The results are shown in Tables I and II.

In *in vitro* tests, HL-60 cells and KB cells were incubated with various concentrations of drugs at 37 °C for 24 h and 3 d, and examined for the inhibition of [^{3}H]thymidine incorporation[21] and by the MTT colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), respectively.[22] All of the eleven compounds (**1a**—**k**) showed concentration-dependent cell growth inhibition (Fig. 1).

Against HL-60 and KB cells, all of the 2-*O*-methyl-3-*O*-(2-trimethylammonioethyl) (**1a**) and corresponding 3-mono- and 3-oligo(ethyleneoxy)ethyl compounds (**1b**—**d**)

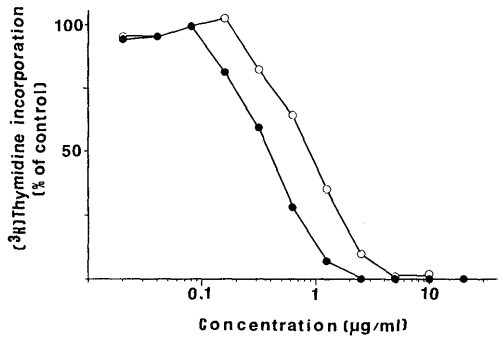


Fig. 1. Dose–Response Curves for Alkyl Glycerolipid on HL-60 Cells (40000 cells/well Incubation, 24-h Alkyl Glycerolipid Treatment, 4-h Incubation with [^{3}H]Thymidine)

○, compound **1b**; ●, compound **1g**.

showed greater *in vitro* antitumor activity than the reference compound, ET18-OMe. This emphasizes the importance of the nonphosphorus oligoether chain which is interposed between the glycerol backbone and the trimethylammonio function. Comparison of the activities among

the compounds (**1a—d**) showed that the most active one was the di(ethyleneoxy)ethyl compound (**1c**) with IC_{50} values of 0.62 and 0.16 μg/ml, respectively, for activity against HL-60 and KB cells. The IC_{50} values of these compounds **1a—d**, were similar, which indicates that at least under the test conditions, the degree of oligomerization of the ethyleneoxy unit in the polarhead side chain plays only a moderate role in the *in vitro* activity.

Compounds **1a—d** were tested for *in vivo* activity by life prolongation assay using mice bearing S180 and mice with mammary carcinoma MM46. When mice were inoculated intraperitoneally (i.p.) with tumor cells and each compound was administered i.p. under the given conditions, definite antitumor activity was observed as shown in Table I. In Table I, the long-term survivors given in parentheses (the numerator) were not included in the calculation of *T/C*. The most potent antitumor activity was shown by the 3-mono(ethyleneoxy)ethyl compound (the ethoxyethyl ether, **1b**), followed by ET18-OMe, the 3-di(ethyleneoxy)ethyl (**1c**), the 3-ethyl (**1a**), and the 3-tetra(ethyleneoxy)ethyl compounds (**1d**). Compound **1d** showed an increased acute toxicity to mice. This is not due to PAF agonistic action, because in contrast to ET18-OMe, all of the ether lipids in this series were confirmed not to cause any aggregation of rabbit platelets at concentrations up to 300 μg/ml, based on determination by the method described in our earlier paper[7] (data not shown). Compound **1b** showed *T/C* values of 394% against S180 and over 400% against MM46 by i.p. administration. In the latter test, all of the treated mice survived over 60 d after tumor inoculation, suggesting complete remission due to the action of **1b**. Consequently, we focused our attention on compound **1b**, because the preferred *in vivo* activity appeared to be associated with the presence of the 3-[2-(2-trimethylammonioethoxy)ethyl] function in the molecule. This prompted us to synthesize and test further 3-[2-(2-trimethylammonioethoxy)ethyl] analogs (**1e—k**) having a variety of 2-substituents, in order to clarify the effect of structural change of the 2-substituent on the *in vivo* activity. Among the compounds listed in Table II, the 2-*O*-pyrimidinyl compound (**1g**) most potently inhibited the growth of tumor cells, particularly HL-60 cells, while the 2-*O*-acetoacetyl compound (**1h**), in which the ester group was apparently metabolizable, was less potent (IC_{50} = 20 μg/ml). Compounds having a 2-*O*-alkyl ether of relatively small size (**1a—g, i—k**) showed activity equivalent to or higher than that of ET18-OMe against HL-60 cells and KB cells. The IC_{50} values of these alkyl ethers (**1a—g, i—k**) were 0.32—2.5 μg/ml and that of ET18-OMe was 0.63—2.5 μg/ml. The results suggest that the functional modifications at the 2-*O*-substituent, when it is small in size and less metabolizable, had some effect on the *in vitro* activity against these tumor cells, though it was not marked.

There was considerable variation in *in vivo* activity (i.p. inoculation). Good results against S180 and MM46 were obtained with compounds **1e—k**, especially with the those possessing a 2-*O*-alkyl ether (**1e,f** and **j**) and the acetonyl ether (**1k**). Interestingly, the compound with a 2-*O*-acetoacetyl group (**1h**) was also very active in contrast with its performance in the *in vitro* test. The 2-*O*-acetonyl compound (**1k**) gave three long-term survivors over 60 d out of five tumor-bearing mice under i.p. administration. Compounds **1g** and **1i** showed moderate activity against S180. The best *in vivo* activity against MM46 was shown by the 2-*O*-trifluoroethyl ether (**1j**), which was comparable to that of the methyl ether (**1b**), followed by the 2-*O*-pyrimidinyl (**1g**), 2-*O*-propyl (**1e**) and carboxymethyl (**1i**) ethers, all of which were superior to the 2-*O*-acetonyl (**1k**), 2-*O*-acetoacetyl compounds (**1h**) and ET18-OMe. The potencies of **1b, e, g, i** and **j** were higher than that of ET18-OMe under the given conditions using these experimental tumor models.

Promising efficacy against solid S180 *in vivo* was shown by the 2-*O*-acetonyl (**1k**), 2-*O*-butyl (**1f**) and 2-*O*-trifluoroethyl compounds (**1j**), with results comparable to that of **1b** (Fig. 2), whether given *p.o.* or i.v. Antitumor activity of **1b** against solid S180 was dose-dependent. These compounds were superior to ET18-OMe. Compound **1h**, in which an ester function is attached to position 2, did not show any significant *in vivo* activity against solid S180 in contrast to its activity against ascitic S180 and MM46. The 2-*O*-carboxymethyl (**1i**) and 2-*O*-pyrimidin-2-yl compounds (**1g**) were potently effective against ascitic MM46 but only moderately active against both ascites and solid S180. The requirement for favorable activity against solid S180 seemed to be a 1-alkyl lysolipid possessing a small and metabolically stable ether at position 2. Tests for antileukemic activity against P388 in mice in comparison with ET18-OMe showed maximal activity against P388 by the 2-*O*-propyl ether (**1e** *T/C* = 169%), followed by the 2-*O*-methyl ether (**1b**, *T/C* = 145%). Other members in this series (**1f, g, h, i** and **k**) showed little or essentially no efficacy under the given experimental conditions.

Fleer *et al.*,[10] on the basis of their observation of a direct correlation between the *in vitro* activity of the ether phospholipids (ET18-OMe and HPC) and their properties as substrates for PLase C and related enzymes, hypothesized that the antitumor activity of these ether lipids arose from their metabolites and that the cytotoxic principles were the corresponding apolar alcohols released from the parent ether lipids by the action of PLase C or related enzymes. In

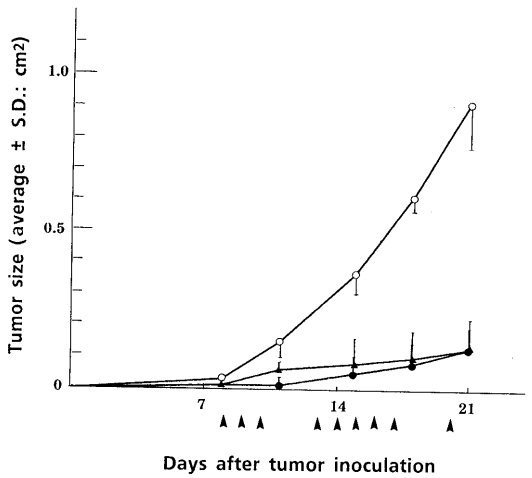


Fig. 2. Retardation of the Growth of Subcutaneously Implanted S180 in Mice by Oral Treatment with Alkyl Glycerolipids, 0.3 mg Daily Nine Times, as Indicated by Arrows

○, nontreated; ●, compound **1b**; ▲, compound **1k**.

order to test the validity of this hypothesis[6,9,10] which has been widely supported, we synthesized and examined the antitumor activity of 1-*O*-octadecyl-2-*O*-methylglycerol[18] which, according to this hypothesis, should be one of such apolar alcohols metabolically formed in tumor cells from ET18-OMe. In the *in vitro* test, this compound exerted some direct effect on HL-60 cells but was far less active than the parent ET18-OMe (the IC_{50} values being 5.5 and 0.42 μg/ml, respectively, for 3-d culture). Our findings showed that the nonphosphorus ether lipids (**1a** and its congeners) characterized by a chemical structure similar to that of ET18-OMe, except for the absence of the phosphoryl group, were potently active against tumors, though they should no longer be substrates for PLases C and D. Recently, Kudo *et al.*, based on their metabolic studies of some ether lipids in HL-60 cells reported that, in contrast to radiolabeled PAF, the nonphosphorus lipid **1b**, tritium-labeled in the *N*-methyl groups of the 3-polar-head side chain, was not degraded at all (no formation of radiolabeled choline or cholinephosphate) after coincubation with HL-60 cells, and hence under the given conditions, this ether lipid was metabolically stable and could not be a substrate for enzymes including these PLases.[23a] In addition, compounds **1a** and **1b** together with other congeners revealed biochemical properties strikingly similar to those of ET18-OMe with regard to the inhibition of phosphocholine biosynthesis.[23b] These findings suggest that, in opposition to the hypothesis of Fleer *et al.*,[10] compound **1b** itself acts as the cytotoxic principle against tumor cells, and in a similar way, ET18-OMe exerts its cytotoxic action not through the liberation of a metabolite (such as an apolar alcohol) but directly.

Recently, numerous tumor cells have been described in which normal maturation can be induced by the addition of diverse agents.[24] Induction of differentiation of tumor cells *in vitro* and *in vivo* should provide a basis for new modes of cancer therapy. We have demonstrated that 1-*O*-tetradecyl-2-*O*-methylglycerophosphocholine and its homologs including ET18-OMe induced myeloid leukemia cells, M1[14,16] and HL-60,[15] to differentiate to macrophages or granulocytes at low concentrations. Similarly, some of the present cationic ether-lipids (*e.g.*, **1g**) were shown to retain the ability to induce cell differentiation of HL-60 cells[25] and of human non-small cell lung adenocarcinoma cells, A549, in *in vitro* and *in vivo* experiments.[23a,26] The ether lipids examined here appear to exert their antitumor activities by killing and causing maturation of tumor cells, both actions of which are believed to be membrane-mediated. The results reported here suggest that the introduction of a 2-pyrimidinyl ether at position 2 can yield analogs with much improved antitumor properties. Such improvements encourage us to pursue further work along these lines.

Experimental

Melting points were determined using a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were measured with a Hitachi 215 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Varian T-60 (60 MHz) or a Varian EM-390 (90 MHz) spectrometer. In the NMR spectra, chemical shifts are given on the δ (ppm) scale with tetramethylsilane as an internal standard, and coupling constants (J) are given in hertz (Hz). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet. All the compounds prepared are racemic.

2-Hydroxy-3-(octadecyloxy)propyl *p*-Toluenesulfonate (3) *p*-Toluenesulfonyl chloride (12.8 g, 67 mmol) was added to a solution of 3-octadecyloxy-1,2-propanediol (**2**, 22.0 g, 64 mmol) and pyridine (24 ml, 0.30 mol) in CH_2Cl_2 (400 ml) and the mixture was stirred at room temperature for 48 h. After addition of ice-water (300 ml), the organic layer was separated, dried over $MgSO_4$ and evaporated to dryness under reduced pressure. The residue was dissolved in Et_2O (200 ml) and the solution was passed through a sintered glass filter precoated with silica gel (22 g). The solvent was evaporated to leave a residue which was dissolved in acetone (200 ml). This solution was cooled. After filtration to remove the precipitates (**2**), the filtrate was evaporated *in vacuo* to give a residue. Hexane (150 ml) was added and the precipitated crystals were collected by filtration, washed with hexane, and dried *in vacuo*. The desired product **3** was obtained as colorless crystals. Yield: 17.4 g (55%). mp 68—69 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.38 (1H, d, J=5 Hz), 2.44 (3H, s), 3.40 (5H, m), 4.03 (3H, m), 7.32 (2H, d, J=8 Hz), 7.80 (2H, d, J=8 Hz). IR (KBr): 3600, 2920, 2850, 1630, 1182, 1130, 1103, 845, 820 cm^{-1}.

2-[2-Hydroxy-3-(octadecyloxy)propyloxy]ethyl Trityl Ether (5a) Sodium hydride (60% dispersion in mineral oil) (16 g, 0.4 mol) was added portionwise to a mixture of **3** (150 g, 0.30 mol), ethylene glycol (220 ml, 2.29 mol) and dioxane (270 ml) at room temperature with vigorous stirring. After constant stirring for 1 h at room temperature, the reaction mixture was heated and kept at 90 °C for 13 h with vigorous stirring, then cooled to room temperature. Et_2O (800 ml) and water (2.0 l) were added, and the mixture was shaken. The aqueous layer was separated and extracted twise with 500 ml of Et_2O. The combined organic layer was washed with five 250 ml portions of water, dried with $MgSO_4$ and evaporated to dryness. Pyridine (500 ml) and trityl chloride (84 g, 0.3 mol) were added to the residue (compound **4a**) and the mixture was stirred at 40 °C for 15 h. After evaporation of solvent, the residue was dissolved in CH_2Cl_2 (1.3 l) and washed with dilute HCl and water successively. The organic layer was separated, dried with $MgSO_4$ and evaporated to dryness. The residue was chromatographed on a column of silica gel (1.5 kg) with hexane–AcOEt (12:1—5:1) as the eluent. The eluate containing the desired product was collected and evaporated to give the ether (**4a**) as a colorless oil. Yield: 78 g (42%). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.50 (1H, d, J=3 Hz), 3.20—3.80 (10H, m), 3.80—4.17 (1H, m), 7.23—7.60 (15H, m). IR (Nujol): 3430, 1455, 1115, 1085, 1075, 760, 700 cm^{-1}.

2-[2-[2-Hydroxy-3-(octadecyloxy)propyloxy]ethoxy]ethyl Trityl Ether (5b) This compound was synthesized as a colorless oil starting from **3** (30 g, 60 mmol) as described for **5a**, except for replacement of ethylene glycol with diethylene glycol (64 g, 0.60 mol). Yield: 16.2 g (40%). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.70 (1H, d, J=3 Hz), 3.17—3.83 (15H, m), 7.17—7.60 (15H, m). IR (film): 3420, 2920, 2850, 1490, 1465, 1445, 1110, 1085, 705 cm^{-1}.

2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethanol (7a) A mixture of **5a** (15.8 g, 25 mmol), methyl *p*-toluenesulfonate (8.1 g, 44 mmol), cetyltrimethylammonium chloride (160 mg, 0.5 mmol), 50% aqueous NaOH (8.0 g, 0.1 mol) and toluene (80 ml) was stirred vigorously at 40 °C for 15 h. After evaporation of the reaction mixture, followed by addition of MeOH (10 ml) the resulting mixture was stirred at 50 °C for 30 min, then evaporated to dryness. The residue was dissolved in Et_2O, and the ether solution, after being washed with water, was concentrated under reduced pressure. MeOH (50 ml), dioxane (100 ml) and 2 N HCl (20 ml) were added to the concentrate, which contained **6a**, and the mixture was refluxed for 5 h. The reaction mixture was evaporated to give a residue which was dissolved with CH_2Cl_2 (150 ml). The solution was washed with water, dried with $MgSO_4$ and evaporated. The residue was chromatographed on a column of silica gel (150 g) with hexane–AcOEt (4:1) as the eluent. Work-up in the usual way afforded the desired alcohol (**7a**) as a colorless oil. Yield: 7.7 g (71%). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.47 (1H, s), 3.37—3.90 (14H, m). IR (film): 3430, 2920, 2850, 1455, 1120, 1090, 1060, 720 cm^{-1}.

2-[2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethoxy]ethanol (7b) In the same way as described above, the title compound was synthesized as a colorless oil from **5b** (10.2 g, 15 mmol). Yield: 6.0 g (92%). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.50 (1H, t, J=3 Hz), 3.40—3.80 (18H, m). IR (film): 3425, 2920, 2850, 1465, 1110, 1065, 720 cm^{-1}.

2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethyl *p*-Toluenesulfonate (9) A mixture of **7a** (7.3 g, 18 mmol), *p*-toluenesulfonyl chloride (5.2 g, 27 mmol), Et_3N (3.0 g, 30 mmol) and toluene (40 ml) was allowed to react for 4 d at room temperature and evaporated *in vacuo* to leave a residue. To this, pyridine (20 ml, 25 mmol) and water (2 ml) were added and the

mixture was evaporated to dryness *in vacuo*. After addition of dilute HCl (100 ml) and toluene–Et_2O (1 : 4) (200 ml) to the residue, the mixture was shaken and the organic layer was separated. The aqueous layer was extracted with Et_2O (100 ml) and the combined organic layer was washed successively with aqueous $NaHCO_3$ solution and water, then dried with $MgSO_4$. The evaporation of solvent *in vacuo* gave the title compound (**9**) as a colorless oil. Yield: 10.2 g (quantitatively). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.27 (32H, s), 2.43 (3H, s), 3.33—3.57 (10H, m), 3.67 (2H, t, J=3 Hz), 4.17 (2H, t, J=3 Hz), 7.37 (2H, d, J=8 Hz), 7.80 (2H, d, J=8 Hz). IR (film): 2910, 2850, 1465, 1365, 1350, 1185, 1170, 1120, 1095, 1015, 925, 815 cm^{-1}.

2-[2-[2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethoxy]ethoxy]-ethanol (7c) A mixture of **9** (3.5 g, 6.3 mmol), diethylene glycol (13 g, 126 mmol), 50% aqueous NaOH (2.4 g, 30 mmol), cetyltrimethylammonium chloride (96 mg, 0.30 mmol), and dioxane (30 ml) was stirred vigorously at 70 °C for 2 h and at 80 °C for 2 h. The reaction mixture was concentrated under reduced pressure and the concentrate was extracted with Et_2O (150 ml). The ethereal solution was washed with water, dried with $MgSO_4$ and evaporated to give a residue. This was chromatographed on a column of silica gel (60 g). Elution with hexane–AcOEt–acetone (3 : 1 : 1) gave fractions containing the desired product. After work-up in the usual way, the alcohol (**7c**) was obtained as a colorless oil. Yield: 2.15 g (71%). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.53 (1H, t, J=3 Hz), 3.37—3.83 (22H, m). IR (film): 3440, 2920, 2855, 1465, 1110 cm^{-1}.

2-[2-[2-[2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethoxy]ethoxy]-ethoxy]ethoxy]ethanol (7d) The title compound was synthesized as a colorless oil starting from **9** (3.5 g, 63 mmol) as described for **7c**, except for replacement of diethylene glycol with tetraethylene glycol (9.8 g, 50 mmol). Yield: 2.6 g (71%). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.60 (1H, t, J=4 Hz), 3.37—3.87 (30H, m). IR (film): 3425, 2920, 2850, 1465, 1110, 1065, 720 cm^{-1}.

2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethyltrimethylammonium Methanesulfonate (1a) Methanesulfonyl chloride (0.76 g, 6.5 mmol) was added to a solution of **7a** (2.0 g, 5.0 mmol) and Et_3N (0.92 ml, 6.8 mmol) in CH_2Cl_2 (50 ml). The reaction mixture was stirred for 2 h at room temperature, washed successively with water, saturated aqueous $NaHCO_3$ and saturated aqueous NaCl, dried with $MgSO_4$, and evaporated *in vacuo*. The residue (**8a**) was dissolved in 20% Me_3N/toluene (20 ml) and the mixture was stirred at room temperature for 3 d and further at 55 °C for 4 h. After evaporation, the residue was chromatographed on a column of silica gel (80 g) with $CHCl_3$–MeOH–H_2O (65 : 25 : 2) as the eluent. Fractions containing the desired product was evaporated to leave a residue, which, after being triturated with Et_2O, gave **1a** as a white powder. Yield: 2.06 g (77%). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, t, J=6 Hz), 1.10—1.90 (32H, m), 2.70 (3H, s), 3.43 (9H, s), 3.30—3.70 (14H, m). IR (KBr): 2915, 2850, 1630, 1465, 1210, 1190, 1120, 1060 cm^{-1}.

2-[2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethoxy]ethyltrimethylammonium Chloride (1b) *p*-Toluenesulfonyl chloride (1.68 g, 8.8 mmol) was added to a solution of **7b** (3.58 g, 8.0 mmol) and Et_3N (0.88 g, 8.8 mmol) in CH_2Cl_2 (32 ml). After constant stirring for 80 h at room temperature, the reaction mixture was washed successively with dilute HCl, saturated aqueous $NaHCO_3$ and water, dried with $MgSO_4$, and evaporated to give a residue of **8b**. This was combined with tetrahydrofuran (THF) (40 ml) and 30% aqueous Me_3N (10 ml) and the resulting mixture was stirred at room temperature for 4 d, then evaporated. The residue was dissolved in CH_2Cl_2, and the solution was washed with dilute HCl. After evaporation, the resulting residue was chromatographed on a column of an anion-exchange resin (Amberlite IRA-410, Cl-form, 0.37—0.44 mm, 100 ml) with MeOH as the eluent. The eluate containing **1b** was collected and worked up as described above gave the desired compound (**1b**) as a colorless solid. Yield: 2.94 g (70%). *Anal.* Calcd for $C_{29}H_{62}ClNO_4 \cdot H_2O$: C, 62.17; H, 11.87; N, 2.50. Found: C, 62.24; H, 12.00; N, 2.48. ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, t, J=6 Hz), 1.27 (32H, s), 3.27 (9H, s), 3.43—4.03 (18H, m). IR (Nujol): 3370, 1110, 955, 720 cm^{-1}.

2-[2-[2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethoxy]ethoxy]ethyltrimethylammonium Chloride (1c) By a procedure similar to that described above, compound **1c** was synthesized from **7c** (2.1 g, 4.4 mmol) as a colorless solid. Yield: 2.42 g (95%). *Anal.* Calcd for $C_{31}H_{66}ClNO_5 \cdot 0.5H_2O$: C, 64.49; H, 11.70; N, 2.43. Found: C, 64.43; H, 11.73; N, 2.25. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.23 (32H, s), 3.37—3.70 (28H, m), 4.00 (3H, s). IR ($CHCl_3$): 3310, 2925, 2860, 1465, 1235, 1110, 1095, 950 cm^{-1}.

2-[2-[2-[2-[2-Methoxy-3-(octadecyloxy)propyloxy]ethoxy]ethoxy]-ethoxy]ethoxy]ethyltrimethylammonium Chloride (1d) In a similar way, the title compound (**1d**) was synthesized from **7d** (3.0 g, 5.3 mmol) as a colorless solid. Yield: 3.1 g (88%). *Anal.* Calcd for $C_{35}H_{74}ClNO_7$: C, 64.04; H, 11.36; N, 2.17. Found: C, 64.03; H, 11.38; N, 2.10. ^{1}H-NMR ($CDCl_3$+CD_3OD) δ: 0.87 (3H, t, J=6 Hz), 1.30 (32H, s), 3.23 (9H, s), 3.33—3.70 (27H, m), 3.83—4.03 (3H, m). IR ($CHCl_3$): 3320, 2920, 2855, 1465, 1095 cm^{-1}.

2-[2-[2-Hydroxy-3-(octadecyloxy)propyloxy]ethoxy]ethanol (4b) Sodium hydride (60% dispersion in mineral oil) (1.0 g, 25 mmol) was added portionwise to a solution of **10** (24.3 g, 74.4 mmol)[20] and diethylene glycol (160 g, 1.51 mol) in dioxane (600 ml) at room temperature and the resulting mixture was stirred at 50 °C for 30 min, then at 110 °C for 5 h, and evaporated. To this residue, Et_2O (300 ml), hexane (300 ml), and 20% aqueous NaCl were added and the mixture was shaken thoroughly. The separated organic layer was washed with three 150 ml portions of 20% aqueous NaCl, dried with $MgSO_4$, and evaporated. The resulting residue was subjected to chromatography on a column of silica gel (80 g). Elution with a gradient of decreasing hexane concentration in AcOEt–acetone (hexane–AcOEt–acetone; 5 : 1 : 1—2 : 1 : 1) gave the eluate containing the desired compound. After work-up, the title compound **4b** was obtained as a colorless solid. Yield: 18.4 g (57%). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.57 (2H, s), 3.37—3.80 (14H, m), 3.83—4.10 (1H, m). IR (film): 3440, 2920, 2855, 1465, 1120, 1070, 720 cm^{-1}.

2-[2-[2-Hydroxy-3-(octadecyloxy)propyloxy]ethoxy]ethyl Methanesulfonate (11) A solution of methanesulfonyl chloride (5.0 g, 43 mmol) in CH_2Cl_2 (50 ml) was added dropwise with stirring to a mixed solution of **4b** (18.4 g, 42.5 mmol), Et_3N (4.5 g, 45 mmol), and CH_2Cl_2 (185 ml) under ice-cooling and the resulting mixture was further stirred for 1 h at the same temperature. Successive washing with dilute HCl and saturated aqueous $NaHCO_3$ and evaporation to dryness gave a residue. This was chromatographed on a column of silica gel (300 g) with hexane–AcOEt–acetone (6 : 1 : 1→3 : 1 : 1) as the eluent. After work-up, **11** was obtained as a colorless solid. Yield: 13.0 g (60%). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.27 (30H, s), 1.55 (2H, m), 2.57 (1H, d, J=5 Hz), 3.03 (3H, s), 3.33—4.10 (13H, m), 4.30—4.43 (2H, m). IR (Nujol): 3370, 1375, 1210, 1190, 1170, 1115, 1045 cm^{-1}.

2-[2-[2-Hydroxy-3-(octadecyloxy)propyloxy]ethoxy]ethyltrimethylammonium Chloride (12) A solution of **11** (5.1 g, 10 mmol) in EtOH (150 ml) and THF (40 ml) was placed in a 300 ml stainless-steel pressure reactor containing 20 ml of 30% aqueous Me_3N solution and the reactor was sealed. The mixture was stirred at 70 °C for 15 h, then cooled. After evaporation, the residue was dissolved in CH_2Cl_2 (200 ml) and EtOH (10 ml). The solution was washed with saturated aqueous NaCl (100 ml) and evaporated to dryness *in vacuo* giving **12** as a colorless solid. Yield: 4.7 g (87%). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.27 (32H, s), 2.60 (1H, s), 3.40—4.17 (24H, m). IR ($CHCl_3$): 3400, 2930, 2860, 1465, 1185, 1110, 1010 cm^{-1}.

2-[2-[3-Octadecyloxy-2-(propyloxy)propyloxy]ethoxy]ethyltrimethylammonium Chloride (1e) A mixture of **12** (2.1 g, 5.0 mmol), 50% aqueous NaOH (2.4 g, 30 mmol), *n*-propyl iodide (2.55 g, 15 mmol), and CH_2Cl_2 (12 ml) was stirred at room temperature for 15 h. The reaction mixture was acidified with concentrated HCl (3 ml) and extracted with CH_2Cl_2. The organic layer was washed with water and evaporated to dryness. The residue was chromatographed on a column of an anion-exchange resin (Amberlite IRA-410, Cl-form, 0.37—0.44 mm, 100 ml) with MeOH as the eluent. The eluate containing the desired product was evaporated to give a residue, which was subjected to chromatography on a column of silica gel (100 g), eluting with $CHCl_3$–MeOH–H_2O (65 : 25 : 2). The eluate containing **1e** was collected and the solvent was removed. Work-up in the usual way gave **1e** as a colorless solid. Yield: 0.85 g (29%). *Anal.* Calcd for $C_{31}H_{66}ClNO_4 \cdot 1.5H_2O$: C, 64.27; H, 12.00; N, 2.42. Found: C, 64.28; H, 12.04; N, 2.37. ^{1}H-NMR ($CDCl_3$+CD_3OD) δ: 0.87 (3H, t, J=6 Hz), 0.90 (3H, t, J=7 Hz), 1.23 (32H, s), 1.33—2.10 (2H, m), 3.27 (9H, s), 3.33—3.73 (15H, m), 3.83—4.02 (2H, m). IR ($CHCl_3$): 3360, 2925, 2865, 1465, 1110 cm^{-1}.

2-[2-[2-Butyloxy-3-(octadecyloxy)propyloxy]ethoxy]ethyltrimethylammonium Chloride (1f) The title compound **1f** was synthesized as a colorless solid starting from **12** (2.1 g, 5.0 mmol) as described for **1e**, except for replacement of *n*-propyl iodide with *n*-butyl iodide (1.8 g, 10 mmol). Yield: 0.75 g (25%). *Anal.* Calcd for $C_{32}H_{68}ClNO_4 \cdot 2H_2O$: C, 63.81; H, 12.05; N, 2.33. Found: C, 64.14; H, 11.77; N, 2.41. ^{1}H-NMR ($CDCl_3$+CD_3OD) δ: 0.87 (3H, t, J=6 Hz), 0.90 (3H, t, J=7 Hz), 1.23 (32H, s), 1.37—1.70 (4H, m), 3.30 (9H, s), 3.33—3.77 (15H, m), 3.83—4.03 (2H, m). IR ($CHCl_3$): 3340, 2920, 2855, 1465, 1240, 1110, 950 cm^{-1}.

2-[2-[3-Octadecyloxy-2-(pyrimidin-2-yloxy)propyloxy]ethoxy]ethyltrimethylammonium Chloride (1g) A mixture of **12** (3.7 g, 7.0 mmol), 2-

chloropyrimidine (2.3 g, 20 mmol), 50% aqueous NaOH (4.8 g, 60 mmol), CH_2Cl_2 (20 ml), and THF (25 ml) was stirred at room temperature for 60 h. The organic solvent was removed *in vacuo* and the resulting aqueous concentrate was adjusted to pH 3 with 6 N HCl. This mixture was extracted with CH_2Cl_2 and the combined extract was evaporated to dryness. The residue was chromatographed on a column of silica gel (120 g), eluting successively with $CHCl_3$–MeOH (9 : 1—65 : 25) and $CHCl_3$–MeOH–H_2O (65 : 25 : 1—65 : 25 : 4). The eluate containing the desired compound was worked up in the usual way to afford **1g** as a colorless solid. Yield: 3.0 g (67%). *Anal.* Calcd for $C_{32}H_{62}ClN_3O_3 \cdot 3H_2O$: C, 61.36; H, 10.94; N, 6.71. Found: C, 61.27; H, 10.59; N, 6.66. ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ: 0.87 (3H, t, J=6 Hz), 1.27 (32H, s), 3.33 (9H, s), 3.37—4.03 (14H, m), 5.43 (1H, quintet, J=6 Hz), 7.00 (1H, t, J=5 Hz), 8.53 (2H, d, J=5 Hz). IR (Nujol): 3350, 1570, 1420, 1330, 1130, 1110 cm^{-1}.

2-[2-[2-Acetoacetyloxy-3-(octadecyloxy)propyloxy]ethoxy]ethyltrimethylammonium Chloride (1h) Diketene (2 ml, 26 mmol) was added dropwise to a solution of **12** (1.5 g, 2.9 mmol) in pyridine (80 ml) at room temperature with stirring and allowed to react at 45 °C for 5 min. Then EtOH (10 ml) was added to decompose unreacted diketene. The solvent was removed by distillation and the residue was chromatographed on a column of silica gel (38 g), eluting with $CHCl_3$–MeOH (4 : 1—2 : 1). Work-up in the usual way afforded the desired compound (**1h**) as a colorless solid. Yield: 1.5 g (86%). *Anal.* Calcd for $C_{32}H_{64}ClNO_6 \cdot 0.8H_2O$: C, 63.14; H, 10.86; N, 2.30. Found: C, 63.23; H, 11.04; N, 2.29. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.23 (32H, s), 2.27 (3H, s), 3.33—3.67 (23H, m), 3.97 (2H, s), 5.17 (1H, m). IR ($CHCl_3$): 3320, 2925, 2855, 1745, 1715, 1245, 1145, 1110, 910 cm^{-1}.

1-Octadecyloxymethyl-2-[2-(2-trimethylammonioethoxy)ethoxy]ethoxyacetate (1i) A mixture of **12** (1.5 g, 2.9 mmol), chloroacetic acid (0.70 g, 11 mmol), 50% aqueous NaOH (4.0 g, 50 mmol), and dioxane (25 ml) was stirred at 50 °C for 40 h and then cooled to room temperature. To the reaction mixture, acetic acid (3.0 g, 50 mmol) was added with stirring under ice-cooling and the whole was evaporated *in vacuo* to give a residue. Aqueous NaCl (100 ml), EtOH (5 ml) and CH_2Cl_2 (200 ml) were added to the residue and the mixture was shaken thoroughly. The organic layer was separated, and the aqueous layer was further extracted with CH_2Cl_2 (100 ml). The combined organic extract was evaporated to dryness under reduced pressure. The residue was chromatographed on a column of a basic anion-exchange resin, Amberlite IRA-410 (OH-form, 0.37—0.44 mm, 100 ml), with MeOH as the eluent. The eluate containing the desired residue was evaporated. Further chromatography was done on a column of an acidic cation-exchange resin, Amberlite CG-50 (H-form, 100—200 mesh, 30 ml), with THF–H_2O (9 : 1) as the eluent. Work-up in the usual way gave the desired compound **1i** as a colorless solid. Yield: 0.61 g (36%). *Anal.* Calcd for $C_{30}H_{61}NO_6 \cdot 2H_2O$: C, 63.46; H, 11.54; N, 2.47. Found: C, 63.08; H, 11.83; N, 2.47. ^{1}H-NMR ($CDCl_3 + CF_3CO_2H$) δ: 0.83 (3H, t, J=6 Hz), 1.23 (30H, s), 1.40—1.77 (2H, m), 3.13 (9H, s), 3.40—3.97 (15H, m), 4.30 (2H, s). IR (Nujol): 3380, 1600, 1115 cm^{-1}.

2-[2-[2-Methanesulfonyloxy-3-(octadecyloxy)propyloxy]ethoxy]ethyl Trityl Ether (13) Methanesulfonyl chloride (0.75 g, 6.5 mmol) was added dropwise to a solution of **5b** (3.4 g, 5.0 mmol) and Et_3N (0.65 g, 6.5 mmol) in CH_2Cl_2 (20 ml) with stirring under ice-cooling. After constant stirring for 1.5 h at the same temperature, the reaction mixture was diluted with hexane (200 ml) and water (100 ml). The organic layer was separated, washed with water, dried with $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on a column of silica gel (100 g) with hexane–AcOEt (5 : 1) as the eluent. Work-up in the usual way afforded **13** as a colorless wax. Yield: 2.7 g (71%). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.27 (32H, s), 3.00 (3H, s), 3.20 (2H, m), 3.37 (2H, m), 3.57—3.80 (10H, m), 4.83 (1H, m), 7.20—7.67 (15H, m).

2-[2-[3-Octadecyloxy-2-(2,2,2-trifluoroethoxy)propyloxy]ethoxy]ethyl Trityl Ether (6j) A mixture of **13** (2.6 g, 3.85 mmol), 2,2,2-trifluoroethanol (2.0 g, 20 mmol), cetyltrimethylammonium chloride (64 mg, 0.20 mmol), 50% aqueous NaOH (0.80 g, 10 mmol), and toluene (7 ml) was stirred at 80 °C for 5 d. After being cooled to room temperature, the reaction mixture was extracted with hexane. The extract was washed with water, dried with $MgSO_4$ and evaporated to dryness *in vacuo*. The residue was chromatographed on a column of silica gel (100 g) with hexane–AcOEt (5 : 1) as the eluent. Work-up in the usual way gave **6j** as a colorless wax. Yield: 1.8 g (67%). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.27 (32H, s), 3.17—3.87 (15H, m), 4.00 (2H, q, J=9 Hz), 7.17—7.57 (15H, m). IR (film): 3060, 3020, 2975, 2860, 1495, 1465, 1450, 1280, 1160, 1120, 1090, 965 cm^{-1}.

2-[2-[3-Octadecyloxy-2-(2,2,2-trifluoroethoxy)propyloxy]ethoxy]ethyltrimethylammonium Chloride (1j) A mixture of **6j** (1.8 g, 2.6 mmol), dioxane (15 ml), MeOH (20 ml), and concentrated HCl (2 ml) was stirred at 75 °C for 1.5 h and then evaporated *in vacuo*. The residue was extracted with Et_2O and the extract was washed with water, dried with $MgSO_4$, and evaporated. The residue was chromatographed on a column of silica gel (80 g) with hexane–AcOEt–acetone (10 : 3 : 2) as the eluent. Work-up in the usual way afforded 0.92 g of 2-[2-[3-(octadecyloxy)-2-(2,2,2-trifluoroethoxy)propyloxy]ethoxy]ethanol (**7j**) as a colorless oil. The product (**7j**) was dissolved in toluene (40 ml) and hexane (10 ml), then Et_3N (0.30 g, 3.0 mmol) and methanesulfonyl chloride (0.345 g, 3.0 mmol) were added with stirring under ice-cooling. The mixed solution was allowed to react for 2 h at room temperature with stirring. The precipitated $Et_3N \cdot HCl$ was removed by filtration and the filtrate was concentrated under reduced pressure. The concentrate was dissolved in THF (10 ml) and EtOH (2 ml), and 30% aqueous Me_3N (3 ml) was added to the solution. The mixture was allowed to react for 130 h at room temperature and evaporated to dryness *in vacuo*. The residue was chromatographed on a column of a basic anion-exchange resin (Amberlite IRA-410, Cl-form, 0.37—0.44 mm, 100 ml) with MeOH as the eluent. The eluate containing the desired product was evaporated to a residue, which was dissolved in CH_2Cl_2. This solution was washed with water, dried with $MgSO_4$ and evaporated. The residue was subjected to chromatography on a column of silica gel (30 g), eluting with $CHCl_3$–MeOH–H_2O (65 : 25 : 2). After work-up, the desired compound **1j** was obtained as a colorless solid. Yield: 0.80 g (47%). *Anal.* Calcd for $C_{30}H_{61}ClF_3NO_4 \cdot 2H_2O$: C, 57.35; H, 10.43; N, 2.23. Found: C, 57.61; H, 10.50; N, 2.46. ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ: 0.87 (3H, t, J=6 Hz), 1.27 (32H, s), 3.20—4.20 (26H, m). IR ($CHCl_3$): 3320, 2925, 2855, 1465, 1275, 1235, 1155, 1110, 965 cm^{-1}.

2-[2-[2-(2,3-Epoxypropyloxy)-3-(octadecyloxy)propyloxy]ethoxy]ethyl Trityl Ether (14) Sodium hydride (60% dispersion in mineral oil; 0.43 g, 11 mmol) was added portionwise to a solution of **5b** (6.75 g, 10 mmol) and epibromohydrin (9.1 g, 66 mmol) in hexane (50 ml) at room temperature. After stirring of the reaction mixture for 15 h, water (25 ml) was added. The organic layer was separated, washed with water and evaporated. The resulting residue was subjected to chromatography on a column of silica gel (80 g). Elution with hexane–AcOEt–acetone (12 : 1 : 1) gave the eluate containing the required compound. After work-up, the title compound **15** was obtained as a colorless oil. Yield: 6.1 g (83%). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.26 (30H, s), 1.50 (2H, m), 2.52 (1H, m), 2.70 (1H, t, J=5 Hz), 3.00—3.93 (18H, m), 7.18—7.55 (15H, m). IR (film): 2925, 2860, 1445, 1110, 1085, 705 cm^{-1}.

2-[2-[2-(2-Hydroxypropyloxy)-3-(octadecyloxy)propyloxy]ethoxy]ethyl Trityl Ether (15) $LiAlH_4$ (0.22 g, 5.8 mmol) was added portionwise to a solution of **14** (6.08 g, 8.2 mmol) in Et_2O (50 ml) with constant stirring under ice-cooling. After 30 min, additional $LiAlH_4$ (0.22 g, 5.8 mmol) was added and the resulting mixture was further stirred for 1 h at the same temperature. THF–H_2O (4 : 1) (5 ml) was added and the precipitate was removed by filtration. The filtrate was evaporated to dryness *in vacuo* to give **15** as a colorless oil. Yield: 6.1 g (quantitative). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=6 Hz), 1.05 (3H, d, J=6 Hz), 1.26 (30H, s), 1.50 (2H, m), 3.13—4.00 (18H, m), 7.17—7.51 (15H, m). IR (film): 2925, 2860, 1445, 1090, 705 cm^{-1}.

2-[2-[3-Octadecyloxy-2-(2-oxopropyloxy)propyloxy]ethoxy]ethyl Trityl Ether (6k) Jones reagent (5.5 ml), which was prepared from CrO_3 (6.0 g), 97% H_2SO_4 (3.6 ml), and H_2O (18 ml), was added dropwise to a solution of **15** (6.08 g, 8.3 mmol) in acetone (100 ml) over a 30 min period at room temperature with stirring. The mixture was allowed to react for 30 min under constant stirring. Isopropanol (2 ml) was added to decompose excess oxidizing agent, and the mixture was stirred for a further 30 min at room temperature and then neutralized with $NaHCO_3$. Insoluble materials were removed by filtration and the filtrate was evaporated to leave a residue. Extraction with Et_2O followed by washing with water, drying with $MgSO_4$ and evaporation gave a residue. This was chromatographed on a column of silica gel (150 g), eluting with hexane–AcOEt–acetone (23 : 1 : 1). After work-up, the desired compound (**6k**) was obtained as a colorless oil. Yield: 2.2 g (37%). ^{1}H-NMR ($CDCl_3 + CD_3OD$) δ: 0.87 (3H, t, J=6 Hz), 1.26 (30H, s), 1.50 (2H, m), 2.07 (3H, s), 3.17—3.71 (15H, m), 4.21 (2H, s), 7.17—7.50 (15H, m). IR (film): 2920, 2850, 1720, 1445, 1090, 705 cm^{-1}.

2-[2-[3-Octadecyloxy-2-(2-oxopropyloxy)propyloxy]ethoxy]ethanol (7k) A mixture of **6k** (2.2 g, 3.0 mmol), dioxane (45 ml), MeOH (30 ml), and 2 N HCl (12 ml) was refluxed for 2 h and then evaporated *in vacuo*. The residue was extracted with Et_2O and the ethereal extract was washed with water, dried with $MgSO_4$, and evaporated. The residue was chromatographed on a column of silica gel (50 g) with hexane–AcOEt–acetone (5 : 1 : 1) as the eluent. Work-up in the usual way afforded **7k** as a

colorless oil. Yield: 1.1 g (75%). ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 0.87 (3H, t, $J=6$ Hz), 1.26 (30H, s), 1.50 (2H, m), 2.17 (3H, s), 3.33—3.73 (15H, m), 4.23 (2H, s), 7.17—7.50 (15H, m). IR (film): 3400, 2920, 2850, 1730, 1445, 1115 cm^{-1}.

2-[2-[3-Octadecyloxy-2-(2-oxopropyloxy)propyloxy]ethoxy]ethyltrimethylammonium Chloride (1k) A mixture of **7k** (2.0 g, 4.1 mmol), *p*-toluenesulfonyl chloride (0.86 g, 4.5 mmol), Et_3N (0.45 g, 4.5 mmol), and THF (10 ml) was stirred at room temperature for 100 h. After addition of 30% aqueous Me_3N solution (5 ml), the combined mixture was stirred at room temperature for 100 h, and evaporated to dryness. The residue was chromatographed on a column of a basic anion-exchange resin, Amberlite IRA-410 (Cl form, 0.37—0.44 mm, 100 ml), with MeOH as the eluent. The eluate containing **1k** was evaporated to dryness, giving a residue. Further chromatography on a column of silica gel (50 g), eluting with MeOH gave **1k** as a colorless wax. Yield: 0.96 g (40%). *Anal.* Calcd for $C_{31}H_{64}ClNO_5 \cdot H_2O$: C, 63.72; H, 11.39; N, 2.40. Found: C, 63.58; H, 11.42; N, 2.43. ^{1}H-NMR ($CDCl_3+CD_3OD$) δ: 0.87 (3H, t, $J=6$ Hz), 1.23 (32H, s), 2.17 (3H, s), 3.23 (9H, s), 3.33—4.03 (15H, m), 4.57 (2H, s). IR (KBr): 3430, 2920, 2860, 1730, 1630, 1470, 1360, 1120, 960, 880, 725 cm^{-1}.

Animals and Tumors Specific pathogen-free female ICR, C3H/HeN and CDF_1 mice (6—8 weeks old) were purchased from Shizuoka Laboratory Animal Center (Hamamatsu, Japan). Cell lines of human promyelocytic leukemia cells, HL-60, and human epidermoid carcinoma cells, KB, were obtained, respectively, from Saitama Cancer Center Research Institute (Saitama, Japan) and from Flow Laboratories Inc. (U.K.). The tumor cells used in *in vivo* tests were S180 obtained from the Institute of Microbial Chemistry (Tokyo, Japan), MM46 from Teikyo University (Kanagawa, Japan) and P388 from the Cancer Institute (Tokyo, Japan). They were maintained in the ascites form.

Growth Inhibition Assays a) Inhibition of [^{3}H]Thymidine Incorporation: The cytotoxic potential of each compound tested was measured on the basis of a decrease of [^{3}H]thymidine incorporation into H1-60 cells. The HL-60 cells were maintained in GIT medium (Wako Pure Chemicals Co.). A 100 μl portion of the tumor suspension (4×10^5 cells/ml) was placed into each well of a microwell plate and each compound (100 μl) was then added at various concentrations. Controls contained 100 μl of the medium instead of the test compound. Plates were incubated at pH 7.2 at 37 °C, in an atmosphere of 5% CO_2 at high humidity for 20 h. All cultures were pulsed for 4 h with 1 μCi of [^{3}H]thymidine per well (5 Ci/mmol; Amersham and Buchler, United Kingdom). The radioactivity of the acid-insoluble fraction was countered with a liquid scintillation counter as described previously.[21]

b) Microculture MTT Colorimetric Assay: The cytotoxic potential of the compounds against KB cells was measured using a vital stain, tetrazolium (Sigma Chem. Co.). KB cells were maintained in MEM supplemented with 10% fetal calf serum. Various concentrations of each compound were incubated with 1×10^5 cells/ml (0.1 ml/well) in 16-mm culture dishes. After 3 d at 37 °C in the presence of 5% CO_2 in air at high humidity, viable cells were determined colorimetrically by a modification of the method of Mosmann.[22]

***In Vivo* Antitumor Activity** a) Life-Span Assay against S180: ICR mice (18—22 g) were inoculated intraperitoneally with 1×10^5 S180 cells and received i.p. a test compound (0.33 mg/mouse, i.p., unless otherwise mentioned) on days 0, 1 and 2 after inoculation. Five mice were used for each test compound. Experiments were terminated on day 60. Control mice, which received saline in place of the test compound, died within 15 d after inoculation of the cells. Antitumor activity was assessed on the basis of % T/C (100 × mean life-span of treated mice/mean life-span of control mice).

b) Tumor Growth Inhibition Test on Solid S180 in Mice: A test sample at a suitable concentration in saline was injected into mice (0.3 mg/0.2 ml/mouse, i.p. or *p.o*, 5 mice/group) 5 times/week for 2 weeks, starting on day 8 after tumor inoculation (s.c., 1×10^6 cells). Mice were kept under observation for 3 weeks, and then killed for final evaluation of the effect of treatment on tumor growth. Inhibition ratios were calculated with the following formula:

$$\text{inhibition ratio } (\%) = [(A-B)/A]\times100$$

where A is the average tumor weight of the control group and B is that of the treated group.

c) Life-Span Assay against Mouse Mammary Carcinoma, MM46: A tumor inoculum of 1×10^4 MM46 cells was transplanted i.p. into C3H/He female mice on day 0. Five mice were used for each test compound (0.25 mg/mouse, i.p.) and each compound was given i.p. once a day on days 2—5. The activity was assessed on the basis of % T/C as described above. In control mice, mortality was 100% within 20 d after transplantation.

d) Life-Span Assay against Mouse Leukemia P388: CDF_1 mice (5 mice/group) were inoculated intraperitoneally with 1×10^6 P388 cells on day 0, and a test compound was administered i.p. (0.5 mg/mouse) on days 0 and 5 after inoculation. Control mice received saline in place of the test compound. The activity was assessed on the basis of % T/C as described above.

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