Potent Immunosuppressants, 2-Alkyl-2-aminopropane-1,3-diols[1]

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Several immunosuppressants, ISP-I [(2*S*,3*R*,4*R*)-(*E*)-2-amino-3,4-dihydroxy-2-(hydroxymethyl)-14-oxoeicos-6-enoic acid, myriocin = thermozymocidin] and mycestericins A−G, were isolated from culture broths of *Isaria sinclairii* and *Mycelia sterilia*, respectively. In order to investigate structure−activity relationships, extensive modifications of ISP-I were conducted, and it was established that the fundamental structure possessing the immunosuppressive activity is a symmetrical 2-alkyl-2-aminopropane-1,3-diol. The tetradecyl, pentadecyl, and hexadecyl derivatives prolonged rat skin allograft survival in the combination of LEW donor and F344 recipient and were more effective than cyclosporin A. Among them, 2-amino-2-tetradecylpropane-1,3-diol hydrochloride, ISP-I-55, showed the lowest toxicity. ISP-I-55 is a promising lead compound for the development of effective immunosuppressants for organ transplantations and for the treatment of autoimmune diseases.

Introduction

Immunosuppressants are clinically important for organ transplantations and the treatment of autoimmune diseases. Since cyclosporin A (CsA)[2] was introduced, the success rate in organ transplantations has increased remarkably. Recently, FK-506[3] was found to be 10−100-fold more potent than CsA as an immunosuppressant. These compounds have very similar mechanisms of action, inhibiting the production of interleukin-2 (IL-2), a signal molecule that induces cytotoxic T cells,[4] but higher doses of both compounds induce renal dysfunction and other side effects.[5] Therefore, less toxic drugs for the prevention of graft rejection are needed.

Recently, we isolated a potent immunosuppressant, ISP-I[6] (**1**) (Figure 1), from the culture broth of *Isaria sinclairii* (ATCC 24400), which is the imperfect stage of *Cordyceps sinclairii*. *Cordyceps* is a genus of fungus which belongs to Hypocreaceae, in the family of Ascomycetes, and is parasitic on insects such as *Lepidoptera adonata*. *Cordyceps sinensis* Sacc. (Chinese name: Dong Chong Xia Cao) has been used in Chinese traditional medicine as a drug for eternal youth. ISP-I is identical with myriocin[7] and thermozymocidin,[8] previously isolated from the culture broths of other fungi, *Myrioccocum albomyces* and *Mycelia sterilia*, respectively, as an antifungal agent. Nevertheless, we were the first to find that **1** is an immunosuppressant. The immunosuppressive activity of **1** was 10−100 times more potent than that of CsA in terms of suppressing both lymphocyte proliferation in mouse allogeneic mixed lymphocyte reaction (MLR) *in vitro* and generation of allo-reactive cytotoxic T lymphocytes in mice *in vivo*.[6] Compound **1** has attracted the attention of many synthetic chemists[9] since we reported that **1** inhibited
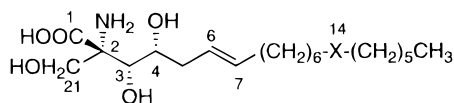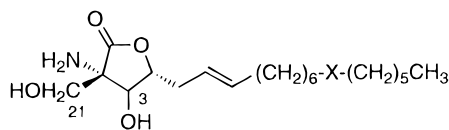

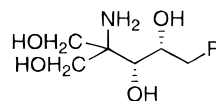**1**: ISP-I (Myriocin, Thermozymocidin) X = CO
2: X = CH$_2$
3: X = CHOH
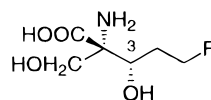
4: Mycestericin D   R= -CH$\overset{E}{=}$CH(CH$_2$)$_6$CO(CH$_2$)$_5$CH$_3$
5: Mycestericin F   R= -(CH$_2$)$_8$CO(CH$_2$)$_5$CH$_3$
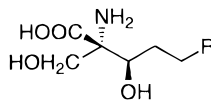
6: Mycestericin E   R= -CH$\overset{E}{=}$CH(CH$_2$)$_6$CO(CH$_2$)$_5$CH$_3$
7: Mycestericin G   R= -(CH$_2$)$_8$CO(CH$_2$)$_5$CH$_3$

8: X= CO   (3*R*)
9: X= CH$_2$ (3*R*)
10: X= CH$_2$ (3*S*)

11: R= -CH$\overset{E}{=}$CH(CH$_2$)$_6$CHOH(CH$_2$)$_5$CH$_3$
12: R= -CH$\overset{E}{=}$CH(CH$_2$)$_{12}$CH$_3$
13: R= -(CH$_2$)$_8$CHOH(CH$_2$)$_5$CH$_3$
14: R= -(CH$_2$)$_{14}$CH$_3$

Figure 1. Structure of ISP-I, mycestericins D−G, and related compounds.

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Scheme 1[a]

R= -CH$_2$CH=CH(CH$_2$)$_{12}$CH$_3$

R$_1$ = TBDMS   R$_2$= Boc
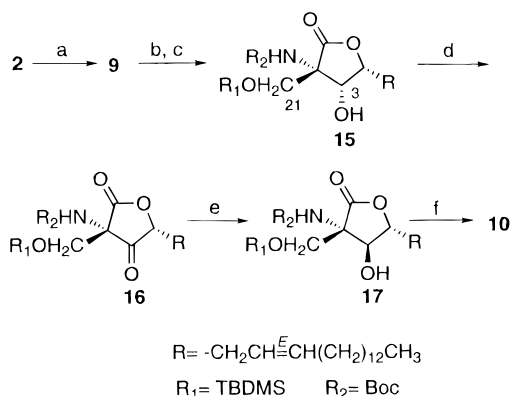
[a] (a) HCl/MeOH; (b) Boc$_2$O, Et$_3$N, DMF; (c) TBDMSCl, imidazole, DMF; (d) PCC, CH$_2$Cl$_2$; (e) NaBH$_4$, MeOH; (f) HCl/MeOH.
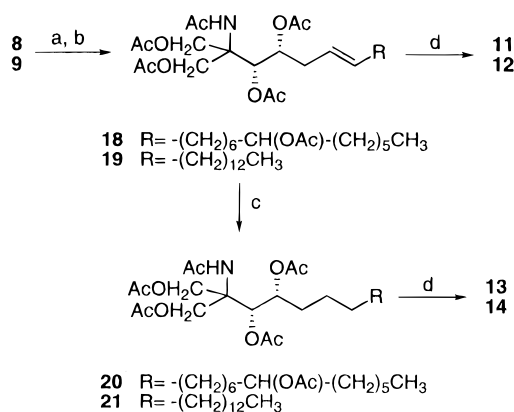
the proliferation of an IL-2-dependent mouse cytotoxic T cell line, CTLL-2, but not the production of IL-2, unlike CsA and FK-506.[6a,10] Very recently, it was revealed that **1** inhibits serine palmitoyltransferase, which catalyzes the committed step of sphingolipid biosynthesis, in CTLL-2[11] and induces apotosis of the cell line.[12]

We have previously reported that functionalities such as the 14-ketone and 6,7-double bond in the side chain of **1** are not essential for the biological activities, including suppression of lymphocyte proliferation in mouse allogeneic MLR.[13] Furthermore, 4-deoxy compounds such as mycestericins D (**4**) and E (**6**), which are minor components of the ISP-I-producing fungus *M. sterilia*, have similar activity to that of **1** on mouse allogeneic MLR, suggesting that the 4-hydroxy group and the configuration of the 3-hydroxy group play no essential role in the biological activities.[14] The dihydro compounds, mycestericins F (**5**) and G(**7**),[14b,c] were less potent than **4** and **6**, but still retained the activity. It appears that the 6,7-double bond is not essential, but may enhance the activity. Therefore, our aim in this work was to identify the fundamental or minimal structure which would retain the biological activities of **1**. Such a structure would be a promising lead for the development of new clinical immunosuppressants.
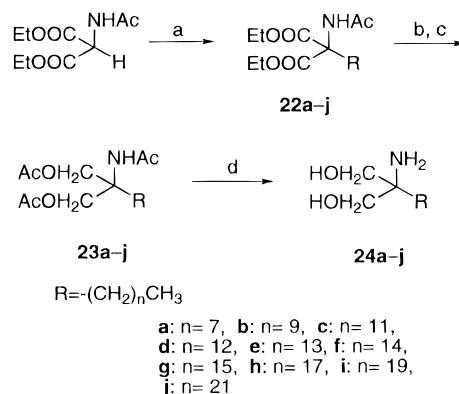
In this paper, we show that simple, symmetric compounds, 2-alkyl-2-aminopropane-1,3-diols, which can be regarded as highly simplified derivatives of ISP-I, have potent immunosuppressive activities.

Chemistry

New ISP-I derivatives **9**−**14** (Figure 1) were synthesized from **1** or 14-deoxoISP-I (**2**).[13] The syntheses of **9** and **10** are shown in Scheme 1. Treatment of **2** with methanolic hydrogen chloride afforded the lactone **9**. The amino and the 21-hydroxy groups of **9** were protected by *tert*-butoxycarbonyl and *tert*-butyldimethylsilyl (TBDMS) moieties, respectively, to give the protected compound **15**. Oxidation of **15** with pyridinium chlorochromate (PCC) in dichloromethane gave the ketone **16**. Reduction of **16** with sodium borohydride afforded the alcohol **17**, which was deprotected with methanolic hydrogen chloride to give the 3-epimer, **10** (d, *J* = 8.3 Hz, 3-H), of **9** (d, *J* = 3.6 Hz, 3-H). The syntheses of **11**−**14** are shown in Scheme 2. Reduction of lactones, **8**[13] and **9**, with lithium aluminum hydride in tetrahydrofuran, followed by acetylation with acetic

Scheme 2[a]

18 R= -(CH$_2$)$_6$-CH(OAc)-(CH$_2$)$_5$CH$_3$
19 R= -(CH$_2$)$_{12}$CH$_3$

20 R= -(CH$_2$)$_6$-CH(OAc)-(CH$_2$)$_5$CH$_3$
21 R= -(CH$_2$)$_{12}$CH$_3$

[a] (a) LiAlH$_4$, THF; (b) Ac$_2$O, pyridine; (c) 5% Pd on C, H$_2$, MeOH; (d) 1 N NaOH(aq), MeOH.

Scheme 3[a]

22a−j

23a−j   **24a−j**

R=-(CH$_2$)$_n$CH$_3$

a: n= 7, **b**: n= 9, **c**: n= 11,
d: n= 12, **e**: n= 13, **f**: n= 14,
g: n= 15, **h**: n= 17, **i**: n= 19,
j: n= 21

[a] (a) R-Br, NaOEt, EtOH; (b) LiAlH$_4$, THF; (c) Ac$_2$O, pyridine; (d) 1 N NaOH(aq), MeOH.

anhydride and pyridine, afforded the acetates **18** and **19**, respectively. These were hydrogenated to give the dihydroacetates **20** and **21**, respectively. The acetates **18**−**21** were hydrolyzed with aqueous sodium hydroxide in methanol to give 2-substituted 2-aminopropane-1,3-diols **11**−**14**, respectively.

Simple 2-alkyl-2-aminopropane-1,3-diols **24a−j** were synthesized *via* the routes outlined in Scheme 3. Diethyl 2-acetamidomalonate was alkylated in the presence of sodium ethoxide in ethanol to afford diethyl 2-acetamido-2-alkylmalonates **22a−j**. Reduction of **22a−j** with lithium aluminum hydride, followed by acetylation, afforded the triacetates **23a−j**, which were hydrolyzed to give 2-alkyl-2-aminopropane-1,3-diols **24a−j**.

Results and Discussion

Various ISP-I derivatives, which were modified in the lipophilic side chain, were evaluated for immunosuppressive activity on mouse allogeneic MLR *in vitro*.[13] Among them, 14-deoxo derivative **2** displayed the most potent activity, and the 14-hydroxy derivative **4**[13] was also potent (Table 1). However, in rat skin allogeneic transplantation with LEW donor and F344 recipient animals *in vivo*, compounds **1**−**3** prolonged the allograft survival insignificantly at a dose up to 0.3 mg/kg ip, and toxicity appeared at the dose of 1 mg/kg (Table 2). We next screened new ISP-I derivatives, which were modified in the hydrophilic part. The amino pentol derivative **11** possessed comparable activity to that of **1** on

Table 1. Effect of **1**−**3**, **9**−**14**, **24a**−**j**, and CsA on Mouse Allogeneic MLR

compd	IC$_{50}$ (nM)	compd	no. of alkyl side chain carbons	IC$_{50}$ (nM)
1	3.0	**24a**	8	3700
2	1.2	**24b**	10	440
3	6.3	**24c**	12	270
9	3.6	**24d**	13	12
10	10	**24e**	14	5.9
11	4.7	**24f**	15	2.9
12	56	**24g**	16	10
13	1630	**24h**	18	12
14	320	**24i**	20	190
CsA	14	**24j**	22	1600

mouse allogeneic MLR (Table 1), suggesting that the carboxyl group is not essential for the activity. The other derivatives, **12**−**14**, were less potent (Table 1). It is, however, interesting that the simplest 14-deoxodihydro derivative **14** still retained the activity. These derivatives **11**−**14** were also evaluated for activity in the rat skin allograft *in vivo* (Table 2). It is noteworthy that **11**−**14** prolonged survival time more effectively than the carboxyl compounds **1**−**3**, and they were approximately 30-fold less toxic than **1**. Although these compounds were inactive when a low dose, 0.3 mg/kg, was given, they were more effective than **1** when the maximal dose, 20−30 mg/kg, was given. It appears that the conversion of the carboxyl group to a hydroxymethyl group of C-1 was effective for increasing the immunosuppressive activity *in vivo* and reducing toxicity. In particular, the 14-deoxy derivatives **12** and **14** prolonged the transplant survival by 2−3 days compared with the corresponding hydroxy compounds **11** and **13** when the maximal dose was given.

The epimeric isomers, mycestericins D (**4**) and E (**6**), at C-3 showed similar activity on mouse allogeneic MLR.[14] There was no great difference between the activities of 14-deoxoISP-I γ-lactone (**9**) and its C-3 epimer **10** on mouse allogeneic MLR (Table 1). Thus, it is suggested that the configuration of the 3-hydroxy group is unimportant. These results indicate that 2-alkyl-2-aminopropane-1,3-diols are the basic structure of ISP-I analogues manifesting immunosuppressive activities. In fact, compound **24h**, having the same carbon skeleton as that of **1**, suppressed the proliferation of lymphocytes in mouse allogeneic MLR, being a quarter as potent as **1** and equipotent to CsA (Table 1). In rat skin allogeneic transplantation, the mean survival time with **24h** was 6 days longer than that with **1**
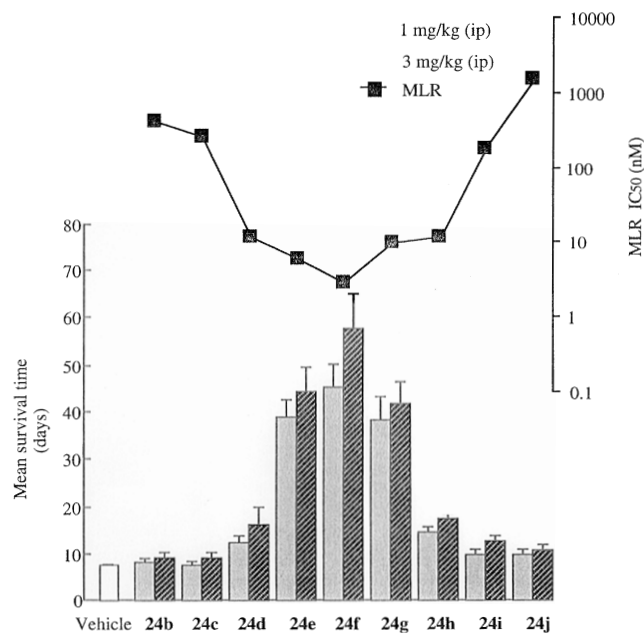
Figure 2. Effect of **24b**−**j** on rat skin allograft and mouse allogeneic MLR.

and was 1−2 days shorter than that with CsA when the maximal dose was given (Table 2).

These results led us to examine the optimization of the side chain length of 2-alkyl-2-aminopropane-1,3-diols to increase the immunosuppressive activity. It was found that 2-alkyl-2-aminopropane-1,3-diols showed a bell-shaped relationship between the immunosuppressive activities and the side chain length. The inhibitory activity on mouse allogeneic MLR *in vitro* and the survival time of rat skin allograft *in vivo* appeared to be well correlated as shown in Figure 2, with the minimum IC$_{50}$ value for the former and the maximum value of the latter at the C$_{15}$ side chain length. The tetradecyl, pentadecyl, and hexadecyl derivatives **24e**−**g** were much more potent than CsA, both *in vitro* (Table 1) and *in vivo* (Table 2). Furthermore, **24e**−**g** (side chain: C$_{14}$−C$_{16}$), as well as **24h** (side chain: C$_{18}$), were approximately 10-fold less toxic than **1**. When the most potent compound *in vitro*, **24f**, and the second most potent compound, **24e**, were examined by oral administration in the rat skin allograft system, both compounds were more effective than CsA (Table 3). However, **24e** was less toxic than **24f**. Compound **24e** caused a substantial reduction in the number of lym-

Table 2. Effect of **1**−**3**, **11**−**14**, **24e**−**h**, and CsA on Rat Skin Allograft (Intraperitoneal Administration)

compd	0.1	0.3	1	3	10	20	30	100 (mg/kg)
				MST $\pm$ SD^a				
1	9.2 $\pm$ 0.8	11.2 $\pm$ 0.5	toxic^b					
2	9.0 $\pm$ 1.0	10.6 $\pm$ 0.8	toxic^b					
3	7.6 $\pm$ 0.5 ns^c	9.6 $\pm$ 0.9	toxic^b					
11			8.2 $\pm$ 0.4 ns^c	9.2 $\pm$ 0.4	11.8 $\pm$ 0.8	13.4 $\pm$ 1.1	toxic^b	
12			8.2 $\pm$ 0.8 ns^c	9.8 $\pm$ 0.8	14.0 $\pm$ 0.7	16.6 $\pm$ 1.1	toxic^b	
13			9.2 $\pm$ 1.3	11.0 $\pm$ 0.7	12.2 $\pm$ 0.8	−	12.5 $\pm$ 0.6	toxic^b
14			8.2 $\pm$ 0.4 ns^c	9.2 $\pm$ 0.4	11.8 $\pm$ 0.8	15.0 $\pm$ 0.7	toxic^b	
24e	11.2 $\pm$ 1.7	23.1 $\pm$ 3.3	39.0 $\pm$ 3.7	44.4 $\pm$ 5.3	toxic^b			
24f	14.0 $\pm$ 3.2	33.5 $\pm$ 23	45.3 $\pm$ 4.9	57.8 $\pm$ 7.2	toxic^b			
24g	10.5 $\pm$ 2.8	14.3 $\pm$ 1.3	38.5 $\pm$ 4.8	41.8 $\pm$ 4.5	toxic^b			
24h		11.2 $\pm$ 1.3	14.8 $\pm$ 0.8	17.6 $\pm$ 1.1	toxic^b			
CsA			7.6 $\pm$ 0.9 ns^c	10.8 $\pm$ 0.4	15.2 $\pm$ 0.8	−	19.4 $\pm$ 1.1	toxic^b

^{a} The graft was inspected daily until rejection, which was defined as more than 90% necrosis of the graft epithelium [$n = 4$−5, $p < 0.01$ (Mann Whitney U test)]. The mean survival time of vehicle-treated grafts is 8.0 days. MST: Mean survival time (days). ^{b} Animals died. ^{c} ns: not significant.

Table 3. Effect of **24e**, **24f**, and CsA on Rat Skin Allograft (Oral Administration)

	MST ± SD[a]						
compd	0.1	0.3	1	3	10	30	100 (mg/kg)
24e	10.3 ± 1.0	13.6 ± 1.1	24.4 ± 4.3	36.4 ± 4.0	46.4 ± 8.3	52.2 ± 10.2	toxic[b]
24f	18.8 ± 2.2	28.5 ± 2.9	35.5 ± 4.8	47.0 ± 5.7	8.3 ± 0.5[d]	toxic[b]	
CsA			8.5 ± 0.6 ns[c]	10.3 ± 1.0	13.5 ± 0.6	20.5 ± 1.7	toxic[b]

[a],[b],[c]See the corresponding footnotes in Table 2. [d] Graft loss without healing.
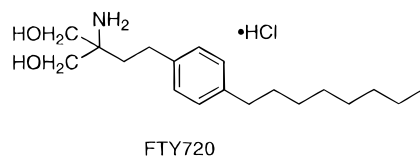
Figure 3. Structure of FTY720.

Table 4. Effect of ISP-I-55 (**24e**) and CsA on IL-2 Production from Alloantigen-Stimulated Mouse Spleen Cells

compd	IC_{50} (nM)
ISP-I-55 (**24e**)	>1000
CsA	10

phocytes in rat peripheral blood [control, 7497 cells/mL; **24e** (3 mg/kg iv), 1613 cells/mL]. The elimination of lymphocytes, such as antigen-primed T cells, is expected to be useful for the prevention of acute rejection in organ transplantations and for the therapy of autoimmune diseases. Consequently, compound **24e**, ISP-I-55, was considered to be optimal among the series as a lead compound for the development of new immunosuppressants. In fact, by the modification of ISP-I-55, we have obtained a novel and potent immunosuppressant, FTY720 [2-amino-2-(4-octylphenyl)ethylpropane-1,3-diol hydrochloride] (Figure 3).[15,16]

We found that ISP-I (**1**) inhibited the proliferation of an IL-2-dependent mouse cytotoxic T cell line, CTLL-2, but not IL-2 production from alloantigen-stimulated T cells.[6a,10] The mechanism of action of **1** appears to be different from that of CsA and FK-506, because CsA and FK-506 inhibited IL-2 production from antigen- or mitogen-stimulated helper T cells.[4] Furthermore, **1** suppressed the proliferation of CTLL-2 cells through the inhibition of serine palmitoyltransferase, which catalyzes ketodihydrosphingosine biosynthesis.[11] Sphingofungin E (**26**),[17] which shows a structural resemblance to **1**, also inhibits the same enzyme action. Therefore, both compounds could exhibit strong binding to this PLP enzyme due to their structural similarity to the proposed reaction intermediate **25**.[18] The growth inhibition induced by **1** was completely abolished by the addition of sphingosine or the 1-phosphate, but not by sphingomyelin or glycosphingolipids.[11] ISP-I-55 (**24e**), like **1**, did not inhibit the production of IL-2 (Table 4). However, **24e** had no inhibitory effect on serine palmitoyltransferase (Figure 4). Furthermore, it was reported that the hydroxy analogue, **27**, of **24e** does not inhibit the incorporation of serine into sphingolipids (Figure 5).[19] Consequently, the structural simplification of ISP-I gave the fundamental structure, 2-alkyl-2-aminopropane-1,3-diol, with the biological activities, but may have altered the point in the biochemical sequence at which the compound intervenes to exhibit immunosuppressive activity.

Conclusion

The 2-alkyl-2-aminopropane-1,3-diol skeleton was identified as the fundamental or minimal structure for
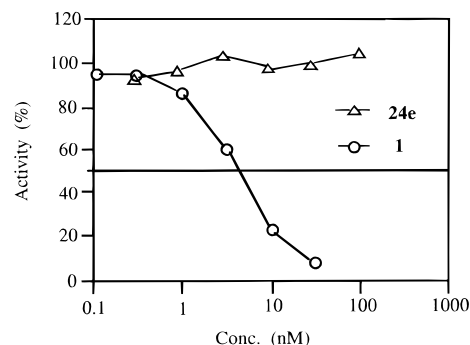
Figure 4. Effect of ISP-I (**1**) and ISP-I-55 (**24e**) on serine palmitoyltransferase activity.

the immunosuppressive activity of ISP-I (**1**). 2-Amino-2-tetradecylpropane-1,3-diol hydrochloride (ISP-I-55, **24e**) was more effective than CsA in the test of skin allograft survival in rats. This derivative seems promising as a lead compound for the discovery of new immunosuppressants for organ transplantations and for the treatment of autoimmune diseases.

Experimental Section

Chemistry. Melting points were determined on a Yanagimoto micro melting point apparatus without correction. IR spectra were taken on a Hitachi 215 infrared spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were taken on JEOL FX-200, JEOL EX-270, and Bruker AC-300 spectrometers with TMS as an internal standard; chemical shifts are reported in parts per million (δ). Mass spectra (FAB-MS) were taken on a JMS-HX110 spectrometer. Analytical high-performance liquid chromatography (HPLC) was performed on a YMC AQ 312 (ODS), 6 × 150 mm or an Asahipak ODP-50, 4.6 × 150 mm column in an oven at 40 °C, with a mobile phase of CH_3-CN/H_2O (80:20), CH_3CN/H_2O (85:15), $MeOH/H_2O$ (85:15), or $CH_3CN/25$ mM Na_2HPO_4 (pH 11) (7:3). The flow rate was set at 1.0 mL/min. On the basis of HPLC analysis, purity ranged from >98.0% to >99.9% for all target compounds analyzed. Preparative HPLC was performed on a YMC-ODS SH-343-5, 20 mm × 250 mm column, with a mobile phase of $CH_3CN/$ H_2O (60:40). The flow rate was set at 9.9 mL/min. For column chromatography on silica gel, Kieselgel 60 (70−230 mesh, Merck) was used. TLC was performed on Kieselgel 60 F_{254} (Merck); compounds were detected by staining with iodine. Where analyses are indicated only by the symbols of the elements, results obtained were within ±0.4% of the theoretical values. Organic solvent extracts were dried over anhydrous magnesium sulfate, and evaporation of solvents was performed under reduced pressure.

14-DeoxoISP-I γ-Lactone (9). A solution of **2**[13] (2.00 g, 5.42 mmol) in MeOH (44 mL) containing concentrated HCl (4 mL) was stirred at 60 °C for 8 h, neutralized with DIAION WA-10, and concentrated to give **9** (1.51 g, 79.2%): mp 102.5−105.0 °C; IR (KBr) 3330, 2930, 2850, 1770, 1570, 1470, 1180, 1120, 1100, 1090, 1060, 1000, 980 cm^{-1}; ^{1}H NMR (200 MHz, CD$_3$OD) δ 5.63 (1H, dtt-like, J = 15.2 and 6.4 Hz, 6-H), 5.48 (1H, dtt-like, J = 15.2 and 6.6 Hz, 7-H), 5.54 (2H, d t, J = 7.1 and 3.6 Hz, 4-H), 4.12 (1H, d, J = 3.6 Hz, 3-H), 3.67 (1H, d, J = 11.2 Hz, 21-Ha), 3.61 (1H, d, J = 11.2 Hz, 21-Hb), 2.45 (2H, t, J = 6.8 Hz, 5-H$_2$), 2.02 (2H, q, J = 6.6 Hz, 8-H$_2$), 1.29 (22H, br s, CH$_2$ × 11), 0.90 (3H, t, J = 6.6 Hz, 20-CH$_3$); ^{13}C NMR (75 MHz, CD$_3$OD) δ 180.59, 135.31, 125.66, 83.40, 73.12, 65.91,
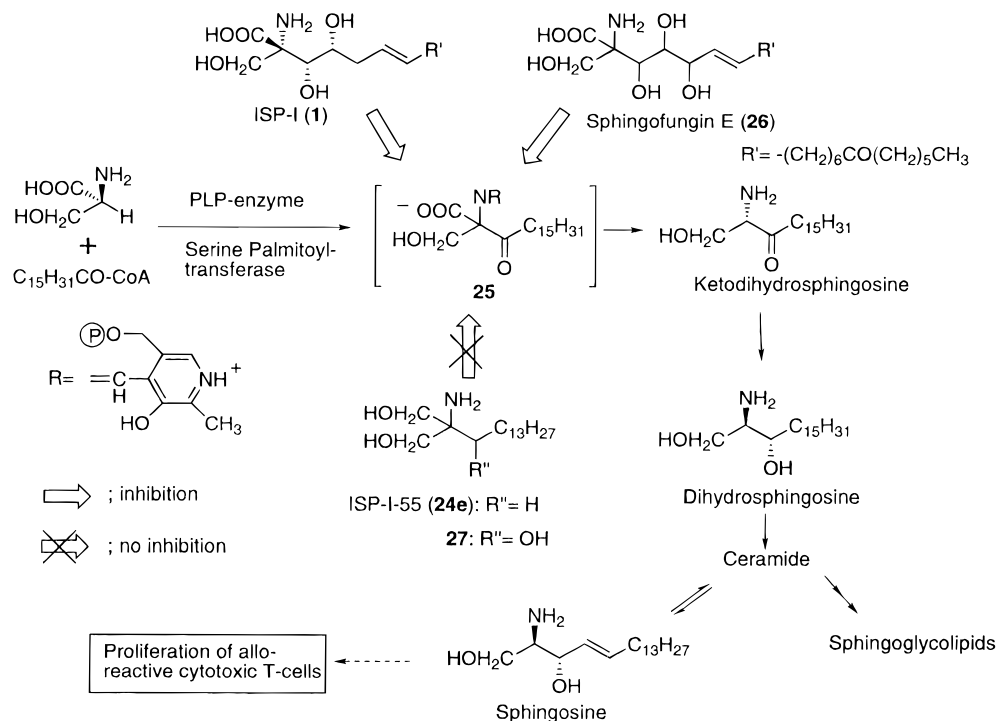
Figure 5. Proposed sites of action of various inhibitors.

65.62, 33.76, 33.17, 33.12, 30.82, 30.66, 30.55, 30.51, 30.31, 23.77, 14.48; MS (FAB) m/z 370 (M + H)$^+$; HRMS (FAB) obsd 370.2954, calcd for $C_{21}H_{40}NO_4$ 370.2959. Anal. ($C_{21}H_{39}NO_4$) H, N; C: calcd, 68.25; found, 67.75.

3-*epi*-14-DeoxoISP-I γ-Lactone (10). A mixture of **9** (1.51 g, 4.09 mmol), di-*tert*-butyl dicarbonate (1.34 g, 6.15 mmol), and a small amount of triethylamine in 15 mL of anhydrous DMF was stirred at room temperature for 12 h. The mixture was poured into water and extracted with EtOAc. The organic solution was washed with saturated brine and dried. The solvent was removed, and the residue was chromatographed on silica gel with *n*-hexane/EtOAc (2:1) to give the *N*-Boc derivative of **9** (1.62 g, 84.4%): IR (KBr) 3400, 3250, 2900, 2850, 1750, 1670, 1540, 1390, 1370, 1300, 1220, 1170, 1040, 1000, 980, 960 cm^{-1}.

A mixture of the *N*-Boc derivative of **9** (1.62 g, 3.45 mmol), *tert*-butyldimethylsilyl chloride (0.780 g, 5.18 mmol), and imidazole (0.705 g, 10.37 mmol) in 10 mL of anhydrous DMF was stirred at 60 °C for 1 h. The mixture was poured into water and extracted with Et$_2$O. The organic solution was washed with saturated brine and dried. The solvent was removed, and the residue was chromatographed on silica gel with *n*-hexane/EtOAc (15:1) to give the *N*-Boc-21-TBDMS ether derivative **15** (1.54 g, 76.2%): IR (CHCl$_3$) 3430, 2930, 2850, 1770, 1690, 1500, 1470, 1370, 1260, 1150, 1120, 1040, 1000, 970, 840 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.63 (1H, dtt-like, $J = 15.3$ and 6.6 Hz, 6-H), 5.46 (1H, dtt-like, $J = 15.4$ and 6.8 Hz, 7-H), 5.26 (1H, br s, NH), 4.57 (1H, dt, $J = 7.1$ and 4.2 Hz, 4-H), 4.53 (1H, m, 3-H), 3.90 (1H, d, $J = 9.7$ Hz, 21-Ha), 3.77 (1H, d, $J = 9.7$ Hz, 21Hb), 2.54 (2H, t, $J = 6.6$ Hz, 5-H$_2$), 2.00 (2H, q, $J = 7.1$ Hz, 8-H$_2$), 1.45 (9H, s, OC(CH$_3$)$_3$), 1.26 (22H, br s, CH$_2$ × 11), 0.88 (9H, s, SiC(CH$_3$)$_3$), 0.88 (3H, t, $J = 6.7$ Hz, 20-H$_3$), 0.06 (6H, s, SiCH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 174.60, 156.57, 134.79, 123.77, 83.36, 81.53, 73.50, 65.90, 65.16, 32.66, 31.94, 31.81, 29.71, 29.68, 29.65, 29.54, 29.37, 29.36, 29.19, 28.21, 25.72, 22.70, 14.12, −5.62, −5.69.

A mixture of **15** (1.54 g, 2.64 mmol), sodium acetate (6.52 g, 79.51 mol), and pyridinium chlorochromate (8.50 g, 39.4 mmol) in 40 mL of anhydrous dichloromethane was stirred under a nitrogen atmosphere at room temperature for 2 h. Et$_2$O and anhydrous MgSO$_4$ were added, and the reaction mixture was stirred for 10 min and then filtered. The residue was washed with Et$_2$O. The combined filtrates were concentrated, and the residue was chromatographed on silica gel with *n*-hexane/EtOAc (10:1) to give the 3-keto compound **16** (0.895

g, 58.5%): IR (CHCl$_3$) 3450, 2930, 2850, 1800, 1780, 1710, 1490, 1470, 1370, 1280, 1260, 1150, 1120, 970, 840 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.64 (1H, dt, $J = 15.3$ and 6.3 Hz, 6-H), 5.53 (1H, dt, $J = 15.4$ and 6.4 Hz, 7-H), 5.03 (1H, br s, NH), 4.58 (1H, dd, $J = 8.0$ and 5.8 Hz, 4-H), 3.91 (1H, d, $J = 8.3$ Hz, 21-Ha), 3.84 (1H, d, $J = 8.3$ Hz, 21-Hb), 2.71 (2H, t, $J = 5.8$ Hz, 5-H$_2$), 2.03 (2H, q, $J = 6.7$ Hz, 8-H$_2$), 1.39 (9H, s, OC-(CH$_3$)$_3$), 1.26 (22H, br s, CH$_2$ × 11), 0.88 (3H, t, $J = 6.9$ Hz, 20-H$_3$), 0.86 (9H, s, SiC(CH$_3$)$_3$), 0.05 (3H, s, SiCH$_3$), 0.03 (3H, s, SiCH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 207.67, 172.72, 154.36, 134.70, 123.71, 85.10, 82.04, 65.34, 61.27, 34.06, 32.58, 31.94, 29.71, 29.68, 29.55, 29.37, 29.26, 29.18, 28.10, 25.63, 22.70, 14.12, −5.86.

Sodium borohydride (70 mg, 1.85 mmol) was added to a solution of **16** (212 mg, 0.365 mmol) in 10 mL of MeOH, and the mixture was stirred for 1.5 h at room temperature, then concentrated. The residue was chromatographed on silica gel with benzene/*n*-hexane (3:1) to give the 3-*epi* compound **17** (168 mg, 79%) of **15**: IR (CHCl$_3$) 3450, 2930, 2850, 1780, 1700, 1500, 1380, 1260, 1160, 1120, 1040, 970, 840 cm^{-1}; ^{1}H NMR (300 MHz, CDCl$_3$) δ 5.60 (1H, dtt-like, $J = 15.3$ and 6.6 Hz, 6-H), 5.44 (1H, dtt-like, $J = 15.9$, 6.8 Hz, 7-H), 5.41 (1H, br s, NH), 4.45 (1H, dt, $J = 7.9$, 4.0 Hz, 4-H), 4.30 (1H, d, $J = 8.5$ Hz, 3-H), 4.17 (1H, d, $J = 9.8$ Hz, 21-Ha), 3.73 (1H, d, $J = 9.8$ Hz, 21-Hb), 2.61 (1H, dt, $J = 14.8$, 5.0 Hz, 5-Ha), 2.35 (1H, dt, $J = 14.9$ and 7.3 Hz, 5-Hb), 2.01 (2H, q, $J = 6.7$ Hz, 8-H$_2$), 1.44 (9H, s, OC(CH$_3$)$_3$), 1.26 (22H, br s, CH$_2$ × 11), 0.88 (9H, s, SiC-(CH$_3$)$_3$), 0.88 (3H, t, $J = 6.8$ Hz, 20-H$_3$), 0.06 (3H, s, SiCH$_3$), 0.05 (3H, s, SiCH$_3$); ^{13}C NMR (75 MHz, CDCl$_3$) δ 173.80, 156.21, 135.09, 123.33, 82.09, 81.25, 78.38, 64.36, 61.96, 35.92, 32.59, 31.94, 29.71, 29.67, 29.63, 29.52, 29.37, 29.33, 29.14, 28.23, 25.72, 22.70, 14.12, −5.58, −5.82.

A solution of **17** (168 mg, 0.288 mmol) in MeOH (3.7 mL) containing concentrated HCl (0.3 mL) was stirred at 60 °C for 4 h, neutralized with DIAION WA-10, and concentrated to give **10** (99 mg, 93%): mp 101.5−103.5 °C; IR (KBr) 3350, 3280, 2930, 2850, 1780, 1600, 1480, 1190, 1120, 1050, 990, 970 cm^{-1}; ^{1}H NMR (300 MHz, CD$_3$OD) δ 5.60 (1H, dt, $J = 15.5$ and 6.4 Hz, 6-H), 5.47 (1H, dt, $J = 15.2$ and 6.6 Hz, 7-H), 4.27 (1H, d, $J = 7.8$ and 4.29 Hz, 4-H), 3.97 (1H, d, $J = 8.3$ Hz, 3-H), 3.87 (1H, d, $J = 10.9$ Hz, 21-Ha), 3.58 (1H, d, $J = 10.6$ Hz, 21-Hb), 2.55 (1H, dt, $J = 14.5$ and 4.8 Hz, 5-Ha), 2.33 (1H, dt, $J = 14.9$ and 7.1 Hz, 5-Hb), 2.03 (2H, q, $J = 6.6$ Hz, 8-H$_2$), 1.28 (22H, br s, CH$_2$ × 11), 0.90 (3H, t, $J = 6.6$ Hz, 20-CH$_3$); ^{13}C NMR (75 MHz, CD$_3$OD) δ 179.96, 135.61, 125.32, 83.16,

79.46, 64.33, 63.97, 37.09, 33.73, 33.12, 30.82, 30.67, 30.55, 30.51, 30.33, 23.77, 14.67; MS (FAB) *m/z* 370 (M + H)⁺; HRMS (FAB) obsd 370.2953, calcd for $C_{21}H_{40}NO_4$ 370.2959.

(3*R*,4*R*)-(*E*)-2-Amino-2-(hydroxymethyl)eicos-6-ene-1,3,4,14-tetrol Hydrochloride (11). Lithium aluminum hydride (800 mg, 21.05 mmol) was added to a stirred solution of **8**[13] (2.00 g, 5.22 mmol) in anhydrous tetrahydrofuran (66 mL) under ice cooling. The reaction mixture was stirred at room temperature for 45 min. Water (1.6 mL) was added dropwise at 0 °C, and the whole was stirred at room temperature for 10 min and then evaporated to dryness. Acetic anhydride (88 mL) and pyridine (80 mL) were added to the residue. The mixture was kept standing overnight, then poured into ice water, and extracted with EtOAc. The organic solution was successively washed with aqueous 1 N HCl, saturated aqueous NaHCO₃, and saturated brine and dried. The organic solvent was removed, and the residue was chromatographed on silica gel with *n*-hexane/EtOAc (6:5) to give the crude acetate (1.667 g). This (650 mg) was purified by preparative HPLC to give the pure acetate **18** (475 mg) as an oil: IR (KBr) 2930, 2860, 1740, 1380, 1240, 1040, 970 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 6.31 (1H, br s, NH), 5.62 (1H, d, *J* = 2.4 Hz, 3-H), 5.48 (1H, dtt-like, *J* = 15.4 and 6.4 Hz, 6-H), 5.26 (1H, dtt-like, *J* = 15.1 and 7.1 Hz, 7-H), 5.13 (1H, dt, *J* = 7.3 and 2.2 Hz, 4-H), 4.85 (1H, q, *J* = 6.1 Hz, 14-H), 4.59 (1H, d, *J* = 11.7 Hz, HOC*H₂*), 4.53 (1H, d, *J* = 11.7 Hz, HOC*H₂*), 4.48 (1H, d, *J* = 11.8 Hz, HOC*H₂*), 4.35 (1H, d, *J* = 11.9 Hz, HOC*H₂*), 2.24 (4H, m, 5- and 8-H₂), 2.16 (3H, Ac), 2.11 (3H, Ac), 2.05 (3H, Ac), 2.04 (3H, Ac), 2.04 (3H, Ac), 1.97 (3H, Ac), 1.52−1.49 (4H, m, 13- and 15-H₂), 1.26 (16H, br s, CH₂ × 8), 0.88 (3H, t, *J* = 6.5 Hz, 20-H₃); ¹³C NMR (75 MHz, CDCl₃) δ 170.94, 170.72, 170.35, 170.17, 170.14, 169.43, 135.15, 123.50, 74.42, 72.13, 71.58, 63.65, 63.41, 60.01, 35.68, 34.15, 32.55, 31.75, 29.40, 29.26, 29.20, 29.09, 25.29, 23.84, 22.58, 21.29, 21.18, 20.73, 20.68, 14.06; MS (FAB) *m/z* 642 (M + H)⁺; HRMS (FAB) obsd 642.3869, calcd for $C_{33}H_{56}NO_{11}$ 642.3856.

A solution of **18** (209 mg, 0.326 mmol) in MeOH (15.6 mL) containing 1 N NaOH (3.9 mL) was refluxed for 6 h. The solution was concentrated, and the residue was dissolved in water. The solution was applied to a SEP-PAK Cartridge C₁₈ (Millipore Co.) and eluted with water and MeOH. The MeOH eluate was acidified with concentrated HCl and concentrated to give compound **11** (104 mg, 75.0%): mp 97.0−98.0 °C; IR (KBr) 3250, 2930, 2850, 1580, 1480, 1080, 1030, 980 cm⁻¹; ¹H NMR (300 MHz, CD₃OD) δ 5.57 (1H, dt, *J* = 15.3 and 6.6 Hz, 6-H), 5.43 (1H, dt, *J* = 15.3 and 6.9 Hz, 7-H), 3.85 (1H, dt, *J* = 6.9 and 1.0 Hz, 4-H), 3.90−3.73 (5H, m), 3.67 (1H, d, *J* = 1.0 Hz, 3-H), 2.31 (2H, t, *J* = 6.7 Hz, 5-H₂), 2.02 (2H, q, *J* = 6.4 Hz, 8-H₂), 1.42−1.31 (20H, m, CH₂ × 10), 0.90 (3H, t, *J* = 6.8 Hz, 20-H₃); ¹³C NMR (75 MHz, CD₃OD) δ 134.99, 126.93, 72.49, 71.95, 69.94, 64.78, 61.38, 60.55, 38.57, 38.49, 38.44, 33.76, 33.08, 30.74, 30.57, 30.56, 30.30, 26.81, 23.72, 14.45; MS (FAB) *m/z* 390 (M + H)⁺, 412 (M + Na)⁺; HRMS (FAB) obsd 390.3210, calcd for $C_{21}H_{44}NO_5$ 390.3221. Anal. ($C_{21}H_{43}$-NO₅HCl) C, H, N.

(3*R*,4*R*)-(*E*)-2-Amino-2-(hydroxymethyl)eicos-6-ene-1,3,4-triol Hydrochloride (12). Lithium aluminum hydride (1.850 g, 48.68 mmol) was added to a stirred solution of **9** (4.492 g, 12.17 mmol) in anhydrous tetrahydrofuran (150 mL) under ice cooling. The reaction mixture was treated in a similar manner to that used for the preparation of **18** to afford the crude triacetate, which was recrystallized from *n*-hexane to give the acetate **19** (3.643 g, 51.3%): mp 80.5−81.5 °C; IR (KBr) 3400, 2930, 2850, 1740, 1680, 1380, 1220, 1040, 970 cm⁻¹; ¹H NMR (270 MHz, CDCl₃) δ 6.30 (1H, br s, NH), 5.61 (1H, d, *J* = 2.3 Hz, 3-H), 5.48 (1H, dtt-like, *J* = 15.2 and 6.6 Hz, 6-H), 5.27 (1H, dtt-like, *J* = 15.2 and 7.3 Hz, 7-H), 5.13 (1H, ddd, *J* = 7.6, 5.9 and 2.3 Hz, 4-H), 4.60 (1H, d, *J* = 11.9 Hz, HOC*H₂*), 4.53 (1H, d, *J* = 11.9 Hz, HOC*H₂*), 4.49 (1H, d, *J* = 11.9 Hz, HOC*H₂*), 4.37 (1H, d, *J* = 11.9 Hz, HOC*H₂*), 2.25− 2.18 (4H, m, 5- and 8-H₂), 2.16 (3H, s, Ac), 2.11 (3H, s, Ac), 2.05 (3H, s, Ac), 2.04 (3H, s, Ac), 1.97 (3H, s, Ac), 1.25 (22H, br s, CH₂ × 11), 0.88 (3H, t, *J* = 6.8 Hz, 20-H₃); ¹³C NMR (67.5 MHz, CDCl₃) δ 170.73, 170.31, 170.19, 170.15, 169.41, 135.31, 123.34, 72.07, 71.55, 63.61, 63.36, 59.91, 35.69, 32.60, 31.91, 29.69, 29.51, 29.36, 29.22, 23.85, 22.70, 21.19, 20.75, 20.68,

14.12; MS (FAB) *m/z* 584 (M + H)⁺; 606 (M + Na)⁺; HRMS (FAB) obsd 584.3806, calcd for $C_{31}H_{54}NO_9$ 584.3801.

A solution of **19** (1.031 g, 1.72 mmol) in MeOH (68.6 mL) containing 1 N NaOH (17.2 mL) was refluxed for 6 h and then concentrated. The residue was washed with water and dissolved in MeOH. The solution was acidified with concentrated HCl and concentrated to afford **12** (621 mg, 85.7%): mp 111.0− 113.5 °C; IR (KBr) 3300, 2930, 2850, 1580, 1510, 1480, 1090, 1080, 1060, 1030 cm⁻¹; ¹H NMR (300 MHz, CD₃OD) δ 5.57 (1H, dtt-like, *J* = 15.2 and 6.5 Hz, 6-H), 5.43 (1H, dtt-like, *J* = 15.3 and 6.9 Hz, 7-H), 3.88−3.73 (5H, m, HOC*H₂* × 2, 4-H), 3.67 (1H, d, *J* = 0.9 Hz, 3-H), 2.31 (2H, t, *J* = 6.7 Hz, 5-H₂), 2.01 (2H, q, *J* = 6.6 Hz, 8-H₂), 1.28 (22H, br s, CH₂ × 11), 0.90 (3H, t, *J* = 6.7 Hz, 20-H₃); ¹³C NMR (67.5 MHz, CD₃OD) δ 135.02, 126.83, 71.93, 69.68, 64.96, 61.13, 60.25, 38.54, 33.80, 33.08, 30.82, 30.67, 30.62, 30.49, 30.35, 23.76, 14.47; MS (FAB) *m/z* 374 (M + H)⁺; HRMS (FAB) obsd 374.3282, calcd for $C_{21}H_{44}NO_4$ 374.3272. Anal. ($C_{21}H_{43}NO_4$HCl) C, H, N, Cl.

(3*R*,4*R*)-2-Amino-2-(hydroxymethyl)eicosane-1,3,4,14-tetrol Hydrochloride (13). A solution of **18** (244 mg, 0.381 mmol) in MeOH (10 mL) was subjected to hydrogenation over 5% palladium carbon (25 mg). The catalyst was filtered off, and the solvent was evaporated to give the dihydro derivative **20** as an oil (167 mg, 68.2%): IR (KBr) 2930, 2860, 1740, 1380, 1240, 1040 cm⁻¹; ¹H NMR (200 MHz, CDCl₃) δ 6.37 (1H, br s, NH), 5.63 (1H, d, *J* = 2.7 Hz, 3-H), 5.12 (1H, ddd, *J* = 7.9, 5.5 and 2.4 Hz, 4-H), 4.85 (1H, qui, *J* = 6.1 Hz, 14-H), 4.59 (1H, d, *J* = 11.5 Hz, HOC*Hₐ*), 4.53 (1H, d, *J* = 11.7 Hz, HOC*Hₐ*), 4.50 (1H, d, *J* = 11.7 Hz, HOC*H_b*), 4.38 (1H, d, *J* = 11.7 Hz, HOC*H_d*), 2.15 (3H, s, Ac), 2.13 (3H, s, Ac), 2.06 (3H, s, Ac), 2.04 (3H, s, Ac), 2.04 (3H, s, Ac), 1.98 (3H, s, Ac), 1.50 (4H, q, *J* = 6.1 Hz, 13- and 15-H₂), 1.26 and 1.24 (24H, s, CH₂ × 12), 0.88 (3H, t, *J* = 6.5 Hz, 20-H₃); ¹³C NMR (67.5 MHz, CDCl₃) δ 170.98, 170.81, 170.55, 170.44, 170.13, 169.52, 74.45, 72.31, 72.06, 63.74, 63.47, 59.87, 34.14, 32.40, 31.75, 29.49, 29.34, 29.22, 25.34, 25.28, 23.85, 22.59, 21.31, 21.22, 20.75, 20.65, 14.09; MS (FAB) *m/z* 644 (M + H)⁺, 666 (M + Na)⁺; HRMS (FAB) obsd 644.4005, calcd for $C_{33}H_{58}NO_{11}$ 644.4012.

A solution of **20** (100 mg, 0.156 mmol) in MeOH (7.5 mL) containing 1 N NaOH (1.87 mL) was refluxed for 6 h. The reaction mixture was treated in a manner similar to that used for the preparation of **11** to give **13** (48 mg, 72.2%): mp 105.5− 107.5 °C; IR (KBr) 3250, 2930, 2850, 1580, 1470, 1060 cm⁻¹; ¹H NMR (270 MHz, CD₃OD) δ 3.86−3.74 (5H, m, 4-H and HOC*H₂* × 2), 3.62 (1H, br s, 3-H), 3.55−3.47 (1H, m, 14-H), 1.60 (2H, m, CH₂), 1.42 and 1.31 (26H, each br s, CH₂ × 13), 0.90 (3H, t, *J* = 6.9 Hz, 20-H₃); ¹³C NMR (67.5 MHz, CD₃OD) δ 72.13, 71.41, 69.88, 64.47, 60.79, 59.89, 38.01, 37.97, 34.81, 32.63, 30.48, 30.37, 30.17, 26.38, 23.32, 14.30; MS (FAB) *m/z* 392 (M + H)⁺, 414 (M + Na)⁺; HRMS (FAB) obsd 392.3369, calcd for $C_{21}H_{46}NO_5$ 392.3378. Anal. ($C_{21}H_{45}NO_5$HCl) H, N, Cl; C: calcd, 58.93; found 58.25.

(3*R*,4*R*)-2-Amino-2-(hydroxymethyl)eicosane-1,3,4-triol Hydrochloride (14). A solution of **19** (621 mg, 1.07 mmol) in MeOH (23 mL) was prepared in the same manner as that used for the preparation of **20** to give the dihydro derivative **21** (290 mg, 46.5%): mp 92.0−93.0 °C; IR (KBr) 3400, 2930, 2850, 1740, 1230, 1040 cm⁻¹; ¹H NMR (270 MHz, CDCl₃) δ 6.38 (1H, br s, NH), 5.62 (1H, d, *J* = 2.6 Hz, 3-H), 5.12 (1H, ddd, *J* = 8.2, 5.6, and 2.6 Hz, 4-H), 4.60 (1H, d, *J* = 11.9 Hz, HOC_a*Hₐ*), 4.54 (1H, d, *J* = 11.9 Hz, HOC_b*Hₐ*), 4.51 (1H, d, *J* = 11.9 Hz, HOC_a*H_b*), 4.38 (1H, d, *J* = 11.9 Hz, HOC_b*H_b*), 2.15 (3H, s, Ac), 2.13 (3H, s, Ac), 2.06 (3H, s, Ac), 2.05 (3H, s, Ac), 1.98 (3H, s, Ac), 1.56−1.43 (2H, m, 5-H₂), 1.25 and 1.24 (28H, s, CH₂ × 14), 0.88 (3H, t, *J* = 6.6 Hz, 20-H₃); ¹³C NMR (67.5 MHz, CDCl₃) δ 170.78, 170.55, 170.40, 170.11, 169.49, 72.29, 72.04, 63.72, 63.45, 59.86, 32.40, 31.91, 29.69, 29.65, 29.61, 29.54, 29.40, 29.36, 25.35, 23.83, 22.70, 21.20, 20.74, 20.63, 14.12; MS (FAB) *m/z* 586 (M + H)⁺. HRMS (FAB) obsd 586.3963, calcd for $C_{31}H_{56}NO_9$ 586.3957.

A solution of **21** (200 mg, 0.342 mmol) in MeOH (14 mL) containing 1 N NaOH (3.42 mL) was refluxed for 6 h and then treated in a manner similar to that used for the preparation of **12** to give **14** (94 mg, 66.7%): mp 119.0−121.5 °C; IR (KBr) 3270, 2920, 2850, 1470, 1080, 1040 cm⁻¹; ¹H NMR (270 MHz, CD₃OD) δ 3.86−3.74 (5H, m, 4-H and HOC*H₂* × 2), 3.62 (1H,

br s, 3-H), 1.28 (30H, $CH_2 \times 15$), 0.90 (3H, t, $J = 6.6$ Hz, 20-H_3); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 71.57, 70.13, 64.73, 60.95, 60.07, 35.08, 32.87, 30.60, 30.28, 26.61, 23.54, 14.38; MS (FAB) m/z 376 (M + H)$^+$, 398 (M + Na)$^+$; HRMS (FAB) obsd 376.3435, calcd for $C_{21}H_{46}NO_4$ 376.342. Anal. ($C_{21}H_{45}NO_4$·HCl) C, H, N, Cl.

2-Amino-2-octadecylpropane-1,3-diol Hydrochloride (24h). Sodium ethoxide (1.71 g, 25.1 mmol) was added to a solution of diethyl acetamidomalonate (5.00 g, 23.2 mmol) in anhydrous ethanol (64 mL). To this mixture was added a solution of 1-bromooctadecane (8.4 g, 25.2 mmol) in anhydrous ethanol (20 mL). The whole was refluxed under a nitrogen atmosphere for 15 h and then neutralized with 1 N HCl solution. After removal of the solvent, the residue was chromatographed on silica gel with *n*-hexane/EtOAc (2:1) to give the alkylated compound **22h** (6.4 g, 59.0%): mp 70−71 °C; IR (KBr) 3260, 2930, 2850, 1740 cm^{-1}; ^{1}H NMR (270 MHz, CDCl$_3$) δ 6.78 (1H, br s, NH), 4.24 (4H, q, $J = 7.2$ Hz, COOCH_2-$CH_3 \times 2$), 2.34−2.28 (2H, m), 2.04 (3H, s, NHCOCH_3), 1.25 (6H, t, $J = 6.93$ Hz, OCH$_2$C$H_3 \times 2$), 1.25 (32H, br s, CH$_2 \times$ 16), 0.88 (3H, t, $J = 6.6$ Hz); ^{13}C NMR (67.5 MHz, CDCl$_3$) δ 168.87, 168.30, 66.59, 62.44, 32.09, 31.95, 29.72, 29.63, 29.52, 29.40, 29.25, 23.61, 23.09, 22.71, 14.14, 14.02; MS (FAB) m/z 470 (M + H)$^+$; HRMS (FAB) obsd 470.3860, calcd for $C_{27}H_{52}$-NO$_5$ 470.3848.

Lithium aluminum hydride (2.560 g, 67.37 mmol) was added to a stirred solution of **22h** (6.4 g, 13.65 mmol) in anhydrous tetrahydrofuran (213 mL) under ice cooling. The reaction mixture was stirred at room temperature for 30 min. Water (5.0 mL) was added dropwise to the mixture at 0 °C, and the whole was stirred at room temperature for 10 min. The solvent was evaporated off, the residue was dissolved in pyridine (80 mL), and acetic anhydride (88 mL) was added to the solution. The mixture was kept standing overnight, then poured into ice water, and extracted with EtOAc. The organic solution was successively washed with aqueous 1 N HCl, saturated aqueous NaHCO$_3$, and saturated brine and dried. The organic solvent was removed, and the residue was chromatographed on silica gel with *n*-hexane/EtOAc (2:1) to give the triacetate **23h** (3.616 g, 56.4%): mp 90−91 °C; IR (KBr) 3380, 2930, 2850, 1750, 1730 cm^{-1}; ^{1}H NMR (270 MHz, CDCl$_3$) δ 5.62 (1H, br s, NH), 4.30 (4H, br s, OCH$_2 \times 2$), 2.08 (6H, s, OAc $\times$ 2), 1.96 (3H, s, NAc), 1.25 (34H, br s, CH$_2 \times$ 17), 0.88 (3H, t, $J = 6.6$ Hz); ^{13}C NMR (67.5 MHz, CDCl$_3$) δ 170.90, 169.99, 64.73, 58.24, 31.95, 31.75, 29.92, 29.72, 29.60, 29.52, 29.38, 24.24, 23.13, 22.71, 20.88, 14.14; MS (FAB) m/z 470 (M + H)$^+$; HRMS (FAB) obsd 470.3839, calcd for $C_{27}H_{52}$-NO$_5$ 470.3848.

A solution of **23h** (3.616 g, 7.71 mmol) in MeOH (94 mL) containing 1 N NaOH (23.1 mL) was refluxed for 6 h, then acidified with 1 N HCl solution, and concentrated. The residue was successively washed with water and *n*-hexane/EtOAc (1:1) to give **24h** (2.311 g, 78.9%): mp 108.5−109.5 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.65 (2H, d, $J = 11.5$ Hz), 3.59 (2H, d, $J = 11.6$ Hz), 1.65 (2H, m), 1.33 (4H, br s), 1.27 (28H, br s), 0.89 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 62.2, 61.6, 32.6, 32.0, 30.7, 30.3, 30.2, 30.1, 30.0, 23.3, 14.3; MS (FAB) m/z 344 (M + H)$^+$; HRMS (FAB) obsd 344.3528, calcd for $C_{21}H_{45}NO_2$ 344.3531. Anal. ($C_{21}H_{45}NO_2$·HCl) C, H, N, Cl.

Compounds **24a−j** were prepared in a manner similar to that used for the preparation of **24h**; their physical data are summarized below.

2-Amino-2-octylpropane-1,3-diol hydrochloride (24a): mp 79.5−81.0 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.66 (2H, d, $J = 11.6$ Hz), 3.60 (2H, d, $J = 11.5$ Hz), 1.65−1.29 (12H, br s), 0.89 (3H, t, $J = 6.6$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 62.0, 61.4, 32.3, 31.8, 30.5, 29.8, 29.7, 23.2, 23.0, 14.2; MS (FAB) m/z 204 (M + H)$^+$; HRMS (FAB) obsd 204.1970, calcd for $C_{11}H_{25}NO_2$ 204.1965. Anal. ($C_{11}H_{25}NO_2$·HCl) C, H, N, Cl.

2-Amino-2-decylpropane-1,3-diol hydrochloride (24b): mp 86.5−89.0 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.68 (2H, d, $J = 11.9$ Hz), 3.60 (2H, d, $J = 11.9$ Hz), 1.65 (2H, m), 1.32 (4H, br s), 1.27 (12H, br s), 0.89 (3H, t, $J = 6.6$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 61.9, 61.3,

32.2, 31.7, 30.4, 29.9, 29.7, 29.6, 23.1, 23.0, 14.2; MS (FAB) m/z 232 (M + H)$^+$; HRMS (FAB) obsd 232.2272, calcd for $C_{13}H_{29}NO_2$ 232.2278. Anal. ($C_{13}H_{29}NO_2$·HCl) C, H, N, Cl.

2-Amino-2-dodecylpropane-1,3-diol hydrochloride (24c): mp 94.0−95.5 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.68 (2H, d, $J = 11.9$ Hz), 3.60 (2H, d, $J = 11.9$ Hz), 1.63 (2H, m), 1.31 (4H, br s), 1.26 (16H, br s), 0.88 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 62.0, 61.3, 32.1, 31.7, 30.3, 29.9, 29.8, 29.7, 29.6, 23.0, 22.9, 14.2; MS (FAB) m/z 260 (M + H)$^+$; HRMS (FAB) obsd 260.2589, calcd for $C_{15}H_{33}NO_2$ 260.2591. Anal. ($C_{15}H_{33}NO_2$·HCl) C, H, N, Cl.

2-Amino-2-tridecylpropane-1,3-diol hydrochloride (24d): mp 103.0−104.5 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.66 (2H, d, $J = 11.6$ Hz), 3.59 (2H, d, $J = 11.5$ Hz), 1.64 (2H, m), 1.33 (4H, br s), 1.28 (18H, br s), 0.89 (3H, t, $J = 6.6$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 62.1, 61.4, 32.4, 31.8, 30.6, 30.1, 30.0, 29.9, 23.2, 23.1, 14.2; MS (FAB) m/z 274 (M + H)$^+$; HRMS (FAB) obsd 274.2752, calcd for $C_{16}H_{35}NO_2$ 274.2748. Anal. ($C_{16}H_{35}NO_2$·HCl) C, H, N, Cl.

2-Amino-2-tetradecylpropane-1,3-diol hydrochloride (24e): mp 96.5−98.5 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.50 (2H, d, $J = 10.9$ Hz), 3.39 (2H, d, $J = 11.2$ Hz), 1.64 (2H, m), 1.31 (4H, br s), 1.26 (20H, br s), 0.88 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 66.7, 65.5, 34.8, 32.1, 30.6, 29.9, 29.6, 23.1, 22.9, 14.2; MS (FAB) m/z 288 (M + H)$^+$; HRMS (FAB) obsd 288.2894, calcd for $C_{17}H_{37}NO_2$ 288.2904. Anal. ($C_{17}H_{37}NO_2$·HCl) C, H, N, Cl.

2-Amino-2-pentadecylpropane-1,3-diol hydrochloride (24f): mp 106.5−108.0 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.67 (2H, d, $J = 11.6$ Hz), 3.60 (2H, d, $J = 11.9$ Hz), 1.64 (2H, m), 1.31 (4H, br s), 1.26 (22H, br s), 0.88 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 62.0, 61.3, 32.2, 31.7, 30.3, 29.9, 29.9, 29.7, 29.6, 23.0, 22.9, 14.2; MS (FAB) m/z 302 (M + H)$^+$; HRMS (FAB) obsd 302.3051, calcd for $C_{18}H_{39}NO_2$ 302.3061. Anal. ($C_{18}H_{39}NO_2$·HCl) C, H, N, Cl.

2-Amino-2-hexadecylpropane-1,3-diol hydrochloride (24g): mp 103.5−105.5 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.68 (2H, d, $J = 11.9$ Hz), 3.60 (2H, d, $J = 11.9$ Hz), 1.64 (2H, m), 1.31 (4H, br s), 1.26 (24H, br s), 0.88 (3H, t, $J = 6.6$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 61.9, 61.3, 32.2, 31.6, 30.3, 29.9, 29.8, 29.7, 29.6, 23.0, 22.9, 14.2; MS (FAB) m/z 316 (M + H)$^+$; HRMS (FAB) obsd 316.3221, calcd for $C_{19}H_{41}NO_2$ 316.3218. Anal. ($C_{19}H_{41}NO_2$·HCl) C, H, N, Cl.

2-Amino-2-eicosylpropane-1,3-diol hydrochloride (24i): mp 112.0−114.5 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.66 (2H, d, $J = 11.6$ Hz), 3.59 (2H, d, $J = 11.5$ Hz), 1.65 (2H, m), 1.33 (4H, br s), 1.27 (32H, br s), 0.89 (3H, t, $J = 6.6$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 62.1, 61.4, 32.4, 31.9, 30.6, 30.2, 29.9, 29.8, 23.2, 23.1, 14.3; MS (FAB) m/z 372 (M + H)$^+$; HRMS (FAB) obsd 372.3854, calcd for $C_{23}H_{49}NO_2$ 372.3844. Anal. ($C_{23}H_{49}NO_2$·HCl) C, H, N, Cl.

2-Amino-2-docosylpropane-1,3-diol hydrochloride (24j): mp 111.0−113.0 °C; IR (KBr) 3300, 2930, 2850 cm^{-1}; ^{1}H NMR (270 MHz, CD$_3$OD) δ 3.59 (2H, d, $J = 11.5$ Hz), 3.65 (2H, d, $J = 11.6$ Hz), 1.65 (2H, m), 1.33 (4H, br s), 1.27 (28H, br s), 0.89 (3H, t, $J = 6.8$ Hz); ^{13}C NMR (67.5 MHz, CD$_3$OD) δ 62.2, 61.6, 32.6, 32.0, 30.7, 30.3, 30.2, 30.1, 30.0, 23.3, 23.3, 14.3; MS (FAB) m/z 400 (M + H)$^+$; HRMS (FAB) obsd 400.4148, calcd for $C_{25}H_{53}NO_2$ 400.4157. Anal. ($C_{25}H_{53}NO_2$·HCl) C, H, N, Cl.

Mouse Allogeneic Mixed Lymphocyte Reaction (MLR).[20] Mouse allogeneic MLR was carried out by culturing BALB/c mouse spleen cells (5×10^5 cells, responder, H-2^d) and an equal number of C57BL/6 mouse spleen cells treated with mitomycin C at 40 mg/mL for 30 min at 37 °C (stimulator, H-2^b) in 200 μL of RPMI 1640 medium containing 5×10^{-5} M 2-mercaptoethanol, 10% fetal calf serum, and a variable amount of test substance. The cells were placed in a 96-well flat-bottomed microtest plate (No. 3072 Falcon, Becton Dickinson, Lincoln Park, NJ) and cultured for 4 days at 37 °C in an atmosphere of 5% CO$_2$. After 96 h, cell proliferation in each well was determined by MTT staining.[21] Results were expressed as IC$_{50}$ values. The IC$_{50}$ values are the mean values

of triplicate determinations. The range of standard errors was less than 10% of the mean values.

Rat Skin Allograft. The effect of compounds on rat skin allograft was examined as follows. The dorsal skin of LEW rats (RT1[l], male, 4 weeks old) was transplanted to the lateral thorax of F344 rats (RT1[lvl], male, 4 weeks old). The compounds were dissolved in 20% (hydroxypropyl)-β-cyclodextrin solution and daily administered intraperitoneally for 10 days beginning on the day of transplantation. When the oral administration route was employed, test compounds were dissolved in 5% (hydroxypropyl)methylcellulose solution. The grafts were inspected daily until rejection, which was defined as more than 90% necrosis of the graft epithelium.

Effect on Interleukin 2 (IL-2) Production by Alloantigen-Stimulated Mouse Spleen Cells. The spleen cells from BALB/c mice were cultured at a concentration of 5×10^6 cells/mL with an equal number of mitomycin C-pretreated spleen cells from C57BL/6 mice in the presence or absence of a test compound in RPMI 1640 medium containing 5×10^{-5} M 2-mercaptoethanol and 10% fetal calf serum. After culturing for 48 h at 37 °C, the culture supernatants were harvested and assayed for their IL-2 activity. IL-2 activity was determined by using IL-2 dependent CTLL-2 cell proliferation assay and expressed in units/mL.[16,22]

Effect on Serine Palmitoyltransferase Activity.[23] The assay mixture contained 100 mM Hepes buffer (pH 8.3 at 25 °C), 2.5 mM EDTA (pH 7.4), 5 mM dithiothreitol, 0.05 mM pyridoxal 5-phosphate, 1.0 mM [^{3}H]serine (20000 cpm/nmol), 0.2 mM palmitoyl-CoA, and 0–3 μM ISP-I or ISP-I-55. A 10 μL aliquot of ISP-I, ISP-I-55, or vehicle (DMSO) was added to 70 μL of the assay buffer, and the reaction was started by the addition of 20 μL of sonicated CTLL-2 cell homogenate (100 μg of protein). A control tube contained all of the added components except palmitoyl-CoA. After incubation for 10 min at 37 °C, 0.2 mL of 0.5 N NH$_4$OH was added and the lipid products were extracted with chloroform. The chloroform layer was transferred to a scintillation vial and dried thoroughly, and then the radioactivity was measured using a Aloka LS 900 liquid scintillation counter.

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