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Efficient Syntheses of a Series of Trehalose Dimycolate (TDM)/ Trehalose Dicorynomycolate (TDCM) Analogues and Their Interleukin-6 Level Enhancement Activity in Mice Sera

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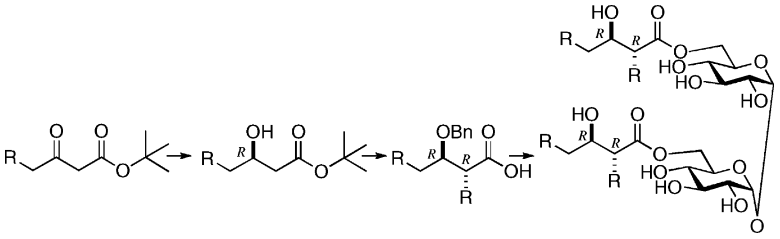

We found an IL-6 level-enhancing compound during our synthetic study of trehalose-6,6′-dimycolate (**1**, TDM, formerly called cord factor) analogues. TDM is a glycolipid distributed in the cell wall of *Mycobacterium tuberculosis* and shows significant antitumor activity based on an immunoadjuvant activity. However, due to its significant toxicity, TDM is not yet applicable for practical use. In 1993, Datta and Takayama reported the purification of trehalose-6,6′-dicorynomycolate (**2c**, TDCM) from *Corynebacterium* spp. We have previously reported the synthesis of four diastereomeric TDCMs and showed that the synthetic (2*R*,3*R*,2′*R*,3′*R*)-TDCM (**2c**, hereafter abbreviated *RRRR*-TDCM−C_{14}) is identical to natural TDCM; we also demonstrated that **2c** and *SSSS*-TDCM−C_{14} (**3c**) showed significant antitumor activity as well as inhibitory activity in experimental lung metastasis based on the immunoadjuvant activity. Furthermore, we found that the significant lethal toxicity in mice by TDM (**1**) was no longer observed with the shorter-chain analogues of TDCMs. Therefore, we have elucidated that the 2,3-antistereochemistry (*RR* or *SS*) of the fatty acid residue is promising for biological activities. The chain length of the fatty acid residue should also be important for the biological activity, and thus, we designed a general synthetic procedure for trehalose diesters with 2,3-antistereochemistry and a series of chain lengths by using Noyori's asymmetric reduction of *β*,*β*-ketoesters followed by antiselective alkylation according to Frater to give *β*,*β*-hydroxy alcohols as the key steps. Thus, we prepared trehalose diesters (TDCM) **2a**−**d**, **3a**−**d**, and **4a**−**d** as well as monoesters (TMCM) **5a**−**d** and **6a**−**d**. Immunological activities of TDCMs and TMCMs were evaluated by determining IL-6 level enhancement in mouse serum, and we found that *RRRR*-TDCM−C_{14} (**2c**) and *RRSS*-TDCM−C_{14} (**4c**) showed significant IL-6 level enhancement activities.

Introduction

Interleukin-6 (IL-6)[1,2] is a multifunctional cytokine exhibiting important roles in host defense, acute phase reaction, immune responses, nerve cell function, and hematopoiesis. Elevated serum IL-6 levels have been observed in a number of pathological conditions, including bacterial and viral infections, trauma, autoimmune diseases and inflammations.[3,4] Although conflicting evidence has reported its involvement in tumor cell growth, the available information about its source and its pathophysiological regulation in cancer cells is limited. While IL-6 has been shown

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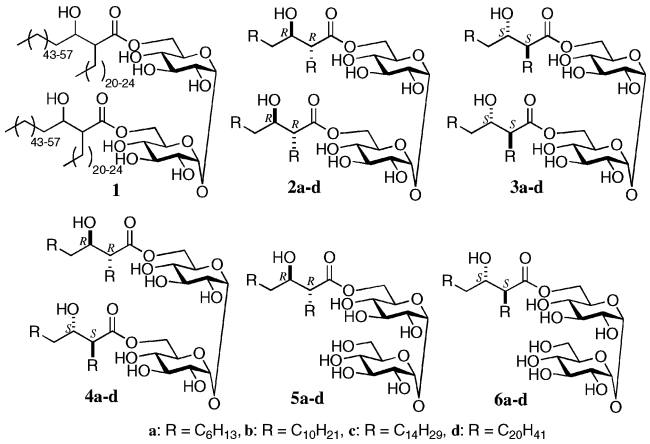
to inhibit the proliferation of some human cancer cell lines and to reduce tumorigenicity in syngeneic animals, it has also been shown to behave as an autocrine growth factor in cases such as multiple myeloma and human prostate cancer.[5] In some autoimmune inflammatory diseases, such as rheumatoid arthritis and Crohn's disease, regulating the function of cytokines may facilitate the restoration of the disequilibrium between pro-inflammatory cytokines and anti-inflammatory cytokines or cytokine inhibitors.[6,7] We have found an IL-6 level-enhancing compound during our synthetic study of trehalose-6,6′-dimycolate (**1**, TDM, formerly called cord factor) analogues. TDM is a glycolipid distributed in the cell wall of *Mycobacterium tuberculosis*[8,9] and shows significant antitumor activity based on an immunoadjuvant activity.[10,11] However, due to its significant toxicity, TDM is not yet applicable for practical use. In 1963, Ioneda reported the existence of smaller glycolipids on the cell surface of related *Corynebacterium diphtheriae*; however, it was shown to be a mixture of three components.[12] In 1993, Datta and Takayama reported the purification of trehalose-6,6′-dicorynomycolate (**2c**, TDCM) from *Corynebacterium* spp.[13] Synthesis of the fatty acid residues of TDCM have been reported, and the stereochemistries were determined to be 2*R*,3*R*.[14] We have reported the synthesis of four diastereomeric TDCMs based on a strategy of the Katsuki−Sharpless epoxidation[15] and then on Noyori's BINAP−Ru-catalyzed asymmetric reduction of a racemic β-ketolactone.[16] Therefore, we showed that the synthetic (2*R*,3*R*,2′*R*,3′*R*)-TDCM (**2c**, hereafter abbreviated *RRRR*-TDCM−C_{14}) is identical to natural TDCM and also demonstrated that **2c** and *SSSS*-TDCM−C_{14} (**3c**) showed significant antitumor activity as well as inhibitory activity in experimental lung metastasis based on the immunoadjuvant activity.[17] Furthermore, we found that the significant lethal toxicity in mice by TDM (**1**) was no longer observed with the shorter-chain analogues of TDCMs.[17] Therefore, we have elucidated that the 2,3-antistereochemistry (*RR* or *SS*) of the fatty acid residue is promising for biological activities. The chain length of the fatty acid residue should also be important for the biological activity, and thus, we designed a general synthetic procedure for trehalose diesters with 2,3-antistereochemistry and a series of chain lengths by using Noyori's asymmetric reduction of β-ketoesters[18] followed by antiselective alkylation according to Frater[19] to give α-alkyl-β-hydroxyesters as the key steps. Thus, we prepared trehalose diesters (TDCM) **2a**−**d**, **3a**−**d**, and **4a**−**d** as well as monoesters (TMCM) **5a**−**d** and **6a**−**d**. Immunological activities of TDCMs and TMCMs were evaluated by determining the IL-6 level enhancement in mouse serum, and we found that *RRRR*-TDCM−C_{14} (**2c**) and *RRSS*-TDCM−C_{14} (**4c**) showed significant enhancement activities.



Results and Discussion

Improved synthesis of natural *RRRR*-TDCM−C_{14} (**2c**) was achieved as follows. A dianion derived from the reaction of *tert*-butyl acetoacetate (**7**) with NaH and then *n*-BuLi was treated with $C_{14}H_{29}I$ in THF to afford **8c** in 78% yield. Exposure to 70 kgf/cm^2 of hydrogen in the presence of $RuCl_2$[(*R*)-BINAP] (0.1 mol %) in MeOH afforded (*R*)-alcohol **9c** in 94% yield. The *R*-stereochemistry of **9c** was confirmed by Kusumi's method,[20] and the optical purity was estimated to be higher than 98% ee based upon the ^{19}F NMR of the MTPA derivative.[21]The dianion derived from reaction of β,β-hydroxyester **9c** with LDA was again treated with $C_{14}H_{29}I$ in the presence of HMPA at −48 °C to provide (*RR*)-**10c** in 55% yield. Diastereoselectivity of the latter alkylation leading to **10c** was confirmed to be higher than 99% de based on ^{1}H NMR as well as HPLC of the derived methyl ester as compared with that of the authentic 2*S*,3*R*-diastereomer.[14,15] Our original reductive etherification of the silylether of **10c** at room temperature gave (*RR*)-benzyl ether carboxylic acid **11c** directly in 96% yield.[22,23] Benzyl-protected

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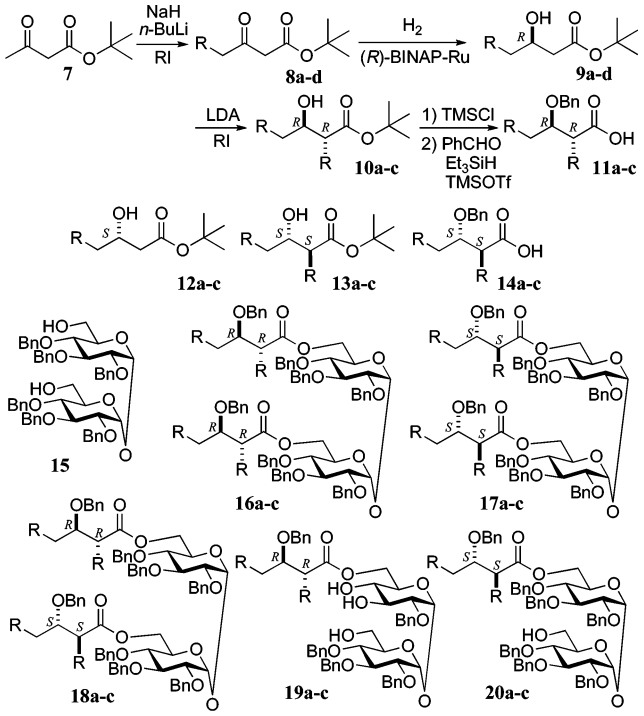
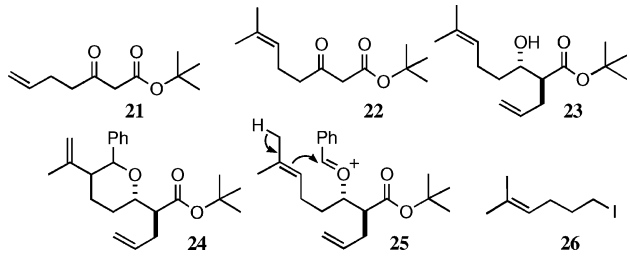
(*SS*)-carboxylic acid **14c** was also prepared by the same procedure, except it utilized (*S*)-BINAP−Ru catalyst for the asymmetric reduction of ketoester **8c** to **12c** and followed the antiselective alkylation to **13c** and reductive etherification/ester cleavage to **14c**.[16,17] Condensation of trehalose derivative **15**[24] and carboxylic acid **11c** (1.5 equiv), carried out using EDCI and DMAP in CH_2Cl_2 at room temperature, afforded diester **16c** in 50% yield along with monoester **19c** in 42% yield.[25] Reaction of monoester **19c** with (*SS*)-acid **14c** (1.2 equiv) under the same conditions gave (*RRSS*)-diester **18c** in 88% yield. Esterification of **15** with (*SS*)-carboxylic acid **14c** (1.5 equiv) using EDCI generated (*SSSS*)-diester **17c** in 48% yield along with (*SS*)-monoester **20c** in 41% yield. Catalytic hydrogenation of esters **16c**, **17c**, **18c**, **19c**, and **20c** using $Pd(OH)_2$ as the catalyst in methanol−chloroform (1:1) afforded *RRRR*-TDCM−C_{14} (**2c**), *SSSS*-TDCM−C_{14} (**3c**), *RRSS*-TDCM−C_{14} (**4c**), *RR*-TMCM−C_{14} (**5c**), and *SS*-TMCM−C_{14} (**6c**), respectively, in 91−97% yields.



a: R = C_6H_{13}, **b**: R = $C_{10}H_{21}$, **c**: R = $C_{14}H_{29}$, **d**: R = $C_{20}H_{41}$

(*RR*)-Carboxylic acids **11a** and **11b** as well as (*SS*)-carboxylic acids **14a** and **14b** were also prepared by the same procedure from ketoester **7**. Carboxylic acids **11a**, **11b**, **14a**, and **14b** were condensed with **15** by the Keck procedure to give **16a**, **16b**, **17a**, **17b**, **18a**, and **18b**,[25] and following catalytic hydrogenation afforded the corresponding TDCM derivatives *RRRR*-TDCM−C_6 (**2a**), *RRRR*-TDCM−C_{10} (**2b**), *SSSS*-TDCM−C_6 (**3a**), *SSSS*-TDCM−C_{10} (**3b**), *RRSS*-TDCM−C_6 (**4a**), and *RRSS*-TDCM−C_{10} (**4b**). TMCM derivatives *RR*-TMCM−C_6 (**5a**), *RR*-TMCM−C_{10} (**5b**), *SS*-TMCM−C_6 (**6a**), and *SS*-TMCM−C_{10} (**6b**) were also prepared via monoesters **19a**, **19b**, **20a**, and **20b**.

When we examined the Frater alkylation of the Noyori reduction product **9d**, which has a very long carbon chain, the alkylation product was not obtained at all probably due to solidification of the material at low temperature. Thus, we switched to the ozonolysis−Wittig strategy for long-chain alkyl derivatives and examined Noyori's asymmetric reduction of allylation product **21**. However, the double bond was also reduced to give the saturated hydroxyester. Prenylation product **22** was efficiently converted to hydroxyester **23** via Noyori reduction and antiselective allylation; however, a cyclization product **24** was obtained under reductive etherification conditions even at low temperature, probably via an oxoniumion intermediate **25**. Finally, we decided to employ bis-homoprenyl iodide **26** for the alkylation of **7**.



Thus, the *tert*-butyl acetoacetate (**7**) was converted to **27** by the reaction of its dianion with iodide **26** in 74% yield. Noyori reduction of **27** using the (*R*)-BINAP−Ru catalyst afforded (*R*)-alcohol **28** in 81% yield. None of the saturated product was detected at all. Dianion generated from **28** with LDA was treated with *n*-$C_{20}H_{41}I$ to give anti-product **29** in 55% yield. Reductive etherification of the TMS ether of **29** at −78 °C afforded benzyl ether *tert*-butyl ester **30** in 96% yield.[22,23] Ozonolysis of **30** and the subsequent Wittig reaction afforded alkene **32**. The *E*/*Z* ratio was not determined. Treatment of **32** with 5 mol % of TMSOTf at room temperature afforded benzyl-protected unsaturated carboxylic acid (*RR*)-**33** in 61% yield in three steps.[22,23] The corresponding (*SS*)-carboxylic acid **39** was prepared by the same procedure except using (*S*)-BINAP−Ru catalyst for the Noyori reduction of **27** to give **34** and successive reactions via **35**, **36**, **37**, and **38**. Condensation of trehalose derivative **15** and (*RR*)-carboxylic acid **33** (1.5 equiv) was carried out using EDCI and DMAP in CH_2Cl_2 at room temperature, affording (*RRRR*)-diester **40** in 44% yield along with (*RR*)-monoester **43** in 41% yield. Reaction of (*RR*)-monoester **43** with (*SS*)-acid **39** (1.2 equiv) under the same conditions gave (*RRSS*)-diester **42** in 88% yield. Esterification of **15** with *SS*-carboxylic acid **39** (1.5 equiv) with EDCI generated (*SSSS*)-diester **41** in 39% yield along with (*SS*)-monoester **44** in 43% yield. Catalytic hydrogenation of esters **40**, **41**, **42**, **43**, and **44** using $Pd(OH)_2$ as the catalyst in ethanol−ethyl acetate afforded *RRRR*-TDCM−C_{20} (**2d**), *SSSS*-TDCM−C_{20} (**3d**), *RRSS*-TDCM−C_{20} (**4d**), *RR*-TMCM−C_{20} (**5d**), and *SS*-TMCM−C_{20} (**6d**), respectively, in 79−92% yield.

With 20 different TDCM analogues in hand, we investigated the immune activation of this class of compounds by evaluating the enhancement of IL-6 levels in mice serum. This assay employs a quantitative sandwich enzyme immunoassay technique.[27] A monoclonal antibody specific for mouse IL-6 was precoated onto a microplate. Standards, controls, and samples were pipetted into the well, and any mouse IL-6 present was

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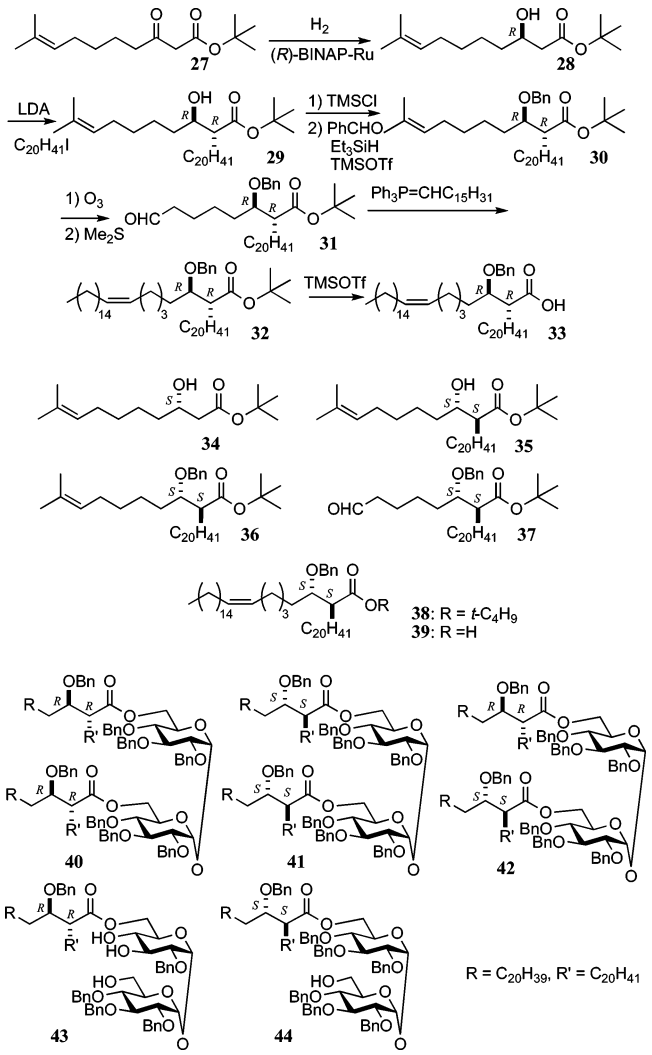

bound by the immobilized antibody. After washing to remove any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-6 was added to the well. Following a wash to remove any unbound antibody−enzyme reagent, a substrate solution was added to the well. The enzyme reaction yields a blue product that turns yellow when the reaction is stopped. The intensity of the color is proportional to the amount of mouse IL-6 bound in the initial step, and the sample values were determined by comparison with a standard curve.

As seen in Table 1, *RRRR*-TDCM−C_{14} (**2c**) induced the highest IL-6 plasma levels, 456.3 ± 160.0 pg/mL, 1 day after its administration; these IL-6 levels were 67-fold higher than those of the control animals. *RRSS*-TDCM−C_{14} (**4c**) also showed significant IL-6 enhancement activity, 195.7 ± 67.1 pg/mL, 1 day after its administration; this corresponds to a 29-fold higher response than that in the control animals. Significant enhancement by **4c** was also observed at day 3. Compounds not listed in Table 1 did not show any IL-6 level enhancement activity. Except for C_{20} analogues **5d** and **6d**, the monoester derivatives were inactive. These results indicate that the TDCM diester analogues effectively and potently enhance IL-6 levels in the plasma of treated animals. The 2,3-antistereochemistry *RR* on the TDCM derivatives together with a precise C_{14} length on the fatty acid residue are structural features necessary for inducing an effective and significant increase of the plasma level of cytokines. Although these results were obtained in healthy animals, the effect of compound **2c** is consistent with previously proposed immunoadjuvant-based antitumor and antimetastasic activities.[17] Moreover, these results afford further evidence for the positive role of IL-6 against tumor development, which has been a point of controversy.[26] Thus, use of TDCMs may advance the knowledge of IL-6 regulation in cancer cells. Based on its nontoxicity, *RRRR*-TDCM−C_{14} (**2c**) certainly constitutes a new potential therapeutic alternative in the immunological treatment of cancer or of other pathological conditions.

We are currently working on the activity of our synthetic TDCM as well as TMCM analogues to other cytokines such as TNF-α and IL-8. We also would like to examine the structure−toxicity relationship of these series of compounds. These results will be published elsewhere.

Experimental Section

***tert*-Butyl-3-oxooctadecanoate (8c).** To a stirred suspension of NaH (847 mg, 35.3 mmol) in THF (100 mL) was dropwise added *tert*-butyl acetoacetate **7** (4.65 g, 29.4 mmol) at 0 °C and stirred for 10 min. Subsequently, *n*-BuLi (1.6 M hexane solution, 20.2 mL, 32.3 mmol) was added, and the mixture was stirred for an additional 30 min at the same temperature. To the resulting mixture was added 1-iodotetradecane (14.3 g, 44.1 mmol), and the mixture was stirred for 30 min at 0 °C. The reaction was quenched by the addition of 1 N HCl solution, and the organic materials were extracted with ether. The dried and concentrated material was subjected to column chromatography on silica gel using hexane and ethyl acetate (20:1) as an eluent to give *tert*-butyl-3-oxooctadecanoate (**8c**) (8.13 g, 78%) as a colorless syrup. **8c**: FT−IR (neat)

TABLE 1. IL-6 Induction on Mice Sera (pg/mL, Mean ± SD)[e]

compound	day 1	day 2	day 3	day 4	day 5
control[a]	6.8 ± 0.8	5.1 ± 1.0	4.0 ± 1.4	7.5 ± 1.6	4.9 ± 1.1
RR-TMCM−C_6 (**5a**)	8.3 ± 1.3	12.0 ± 2.1[c]	6.9 ± 0.8	4.7 ± 0.8	10.0 ± 1.6[b]
SS-TMCM−C_6 (**6a**)	8.1 ± 1.6	10.5 ± 2.9[c]	3.5 ± 0.5	7.5 ± 1.7	9.6 ± 3.5
RRRR-TDCM−C_{10} (**2b**)	37.8 ± 13.7[c]	13.6 ± 3.8	40.7 ± 21.0[c]	40.3 ± 25.2[b]	8.6 ± 2.6
SSSS-TDCM−C_{10} (**3b**)	87.2 ± 23.6[d]	13.2 ± 2.1	13.6 ± 4.7	19.2 ± 3.9	15.9 ± 2.6
RRSS-TDCM−C_{10} (**4b**)	8.9 ± 1.8	13.6 ± 2.9	91.2 ± 26.8[d]	26.4 ± 8.8	24.3 ± 4.4
RRRR-TDCM−C_{14} (**2c**)	456.3 ± 160.0[d]	26.2 ± 12.7[c]	26.9 ± 9.4[c]	71.6 ± 9.4[d]	25.8 ± 4.9[c]
SSSS-TDCM−C_{14} (**3c**)	144.3 ± 9.4[d]	28.8 ± 5.4[d]	87.0 ± 25.5[d]	75.2 ± 13.0[d]	28.6 ± 8.1[c]
RRSS-TDCM−C_{14} (**4c**)	195.7 ± 67.7[d]	15.0 ± 1.6	64.8 ± 21.5[d]	141.7 ± 7.0[d]	24.6 ± 2.9
RRRR-TDCM−C_{20} (**2d**)	17.3 ± 0.6[c]	7.7 ± 2.9	4.2 ± 1.8	14.2 ± 1.5	6.6 ± 1.8
SSSS-TDCM−C_{20} (**3d**)	6.8 ± 2.3	23.4 ± 8.0[c]	2.8 ± 1.3	3.6 ± 0.8	3.0 ± 1.0
RRSS-TDCM−C_{20} (**4d**)	33.5 ± 8.5	41.0 ± 16.4	16.8 ± 4.2	24.8 ± 5.0	15.4 ± 2.5
RR-TMCM−C_{20} (**5d**)	11.0 ± 3.2	41.8 ± 18.9[c]	4.0 ± 3.0	19.2 ± 8.1	13.2 ± 3.6
SS-TMCM−C_{20} (**6d**)	7.6 ± 1.6	16.9 ± 4.9[c]	14.6 ± 5.6[c]	16.3 ± 3.0[c]	13.3 ± 2.7[b]

[a] Control corresponds to mice injected with vehicle and maintained under the same condition as the treated mice. [b] $p < 0.05$. [c] $p < 0.01$. [d] $p < 0.005$. [e] Endogenous level of IL-6: 5−10 pg/mL.

2935, 2858, 1740 cm^{-1}; ^1H NMR (300 MHz in $CDCl_3$) δ 0.88 (3H, t, J = 6.9 Hz), 1.25 (22H, m), 1.47 (9H, s), 1.58 (4H, m), 2.51 (2H, t, J = 7.5 Hz), 3.33 (2H, s); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.1, 22.6, 23.4, 27.9, 29.0, 29.3, 29.4 (many), 29.6 (2C), 31.9, 42.9, 50.6, 81.7, 166.5, 203.4; CIMS *m*/*z* (%) 355 (2, $M^+ + H^+$), 339 (20), 299 (100); HRMS (CI^+) *m*/*z* calcd for $C_{22}H_{43}O_3$ ($M^+ + 1$), 355.3212; found, 355.3215.

(*R*)-*tert*-Butyl-3-hydroxyoctadecanoate (9c). β-Ketoester **8c** (1.78 g, 5.02 mmol) and dichloro[(*R*)-(+)-2,2′-bis(diphenylphosphino)-1,1′-binaphthyl]ruthenium(II) (hereafter abbreviated to (*R*)-BINAP−$RuCl_2$) (3.97 mg, 0.50 μmol) were dissolved in MeOH (3 mL) and deoxygenated by the freeze/melt method under an argon atmosphere. The mixture was stirred for 72 h under H_2 (70 kgf/cm^2) using a TAIATSU SUS316 microautoclave. The concentrated mixture was subjected to column chromatography on silica gel using hexane and ethyl acetate (25:2) as an eluent to give (*R*)-*tert*-butyl-3-hydroxyoctadecanoate (**9c**) (1.68 g, 94%) as a colorless syrup. **9c**: $[\alpha]^{19}_D$ −15.2 (*c* 1.1, $CHCl_3$); FT−IR (neat) 3452, 2923, 2851, 1718 cm^{-1}; ^1H NMR (300 MHz in $CDCl_3$) δ 0.88 (3H, t, J = 6.9 Hz), 1.25 (24H, m), 1.42 (4H, m), 1.47 (9H, s), 2.31 (1H, dd, J = 16.2 and 8.7 Hz), 2.43 (1H, dd, J = 16.2 and 3.3 Hz), 3.12 (OH, d, J = 3.6 Hz), 3.95 (1H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.1, 22.6, 25.4, 28.0, 29.3, 29.5, 29.6 (many), 31.9, 36.4, 42.3, 68.0, 81.0, 172.5; CIMS *m*/*z* (%) 357 (1, $M^+ + H^+$), 343 (5), 301 (100), 283 (55); HRMS (CI^+) *m*/*z* calcd for $C_{22}H_{45}O_3$ ($M^+ + H^+$), 357.3369; found, 357.3383.

(2*R*,3*R*)-*tert*-Butyl-3-hydroxy-2-tetradecyloctadecanoate (10c). To a stirred solution of diisopropylamine (668 mg, 6.60 mmol) in THF (5 mL) was added MeLi (2.2 M ether solution, 2.95 mL, 6.50 mmol) at −78 °C and stirred at 0 °C for 30 min. Then the mixture was cooled to −48 °C, and a solution of **9c** (713 mg, 2.00 mmol) in THF (2 mL) was added. After stirring for 30 min at the same temperature, HMPA (2 mL) and a solution of 1-iododecane (973 mg, 3.00 mmol) in THF (2 mL) were added, and the mixture was stirred for 6 h at −48 °C. To this was added saturated NH_4Cl, which was then extracted with ether. The dried and concentrated extract was subjected to column chromatography on silica gel using hexane and ethyl acetate (20:1) as an eluent to give (2*R*,3*R*)-*tert*-butyl-3-hydroxy-2-tetradecyloctadecanoate (**10c**) (607 mg, 55%) as a colorless syrup. **10c**: $[\alpha]^{20}_D$ +5.3 (*c* 1.0, $CHCl_3$); FT−IR (neat) 3493, 2925, 2853, 1730, 1708 cm^{-1}; ^1H NMR (300 MHz in $CDCl_3$) δ 0.88 (6H, t, J = 6.9 Hz), 1.25 (52H, m), 1.47 (9H, s), 1.62 (2H, m), 2.29 (1H, dt, J = 9.0 and 5.1 Hz), 2.65 (OH, d, J = 8.4 Hz), 3.59 (1H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.1, 22.7, 25.8, 27.3, 28.1, 29.4, 29.5 (2C), 29.6, 29.7 (many), 29.8, 31.9, 35.8, 51.2, 72.5, 81.0, 175.3; CIMS *m*/*z* (%) 554 (5, $M^+ + H^+$), 498 (70), 367 (100), 285 (55), 256 (60); HRMS (CI^+) *m*/*z* calcd for $C_{36}H_{73}O_3$ ($M^+ + H^+$), 553.5560; found, 553.5531.

(2*R*,3*R*)-3-Benzyloxy-2-tetradecyloctadecanoic Acid (11c). To a solution of **10c** (553 mg, 1.00 mmol) in CH_2Cl_2 (3 mL) were added Et_3N (304 mg, 3.00 mmol) and TMSCl (162 mg, 1.50 mmol), and the mixture was stirred for 30 min at room temperature. After addition of brine, the organic materials were extracted with CH_2Cl_2. The dried and concentrated extract was further dried by azeotropic reflux with CH_2Cl_2 using a Soxhlet-type apparatus passing through a 4 Å molecular sieves column, and it cooled to −78 °C. To the mixture were successively added benzaldehyde (159 mg, 1.50 mmol), triethylsilane (174 mg, 1.50 mmol), and trimethylsilyltriflate (111 mg, 50.0 mmol). After stirring for 15 min at −78 °C and then at room temperature for 2 h, the reaction was quenched by the addition of saturated $NaHCO_3$, and the organic materials were extracted with ether. The dried and concentrated extract was subjected to column chromatography on silica gel using hexane and ethyl acetate (10:1) to give (2*R*,3*R*)-3-benzyloxy-2-tetradecyloctadecanoic acid (**11c**) (562 mg, 96%) as a colorless syrup. **11c**: $[\alpha]^{19}_D$ +1.0 (*c* 1.0, $CHCl_3$); FT−IR (neat) 3087, 3065, 3031, 2931, 2854, 1707 cm^{-1}; ^1H NMR (300 MHz in $CDCl_3$) δ 0.88 (6H, t, J = 7.2 Hz), 1.25 (50H, m), 1.60 (4H, m), 2.65 (1H, dt, J = 10.2 and 5.7 Hz), 3.64 (1H, q, J = 6.0 Hz), 4.52 (1H, d, J = 11.4 Hz), 4.60 (1H, d, J = 11.4 Hz), 7.24−7.36 (5H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.1, 22.7, 24.7, 27.6, 29.4, 29.6 (2C), 29.7 (many), 31.0, 31.9, 49.7, 72.0, 79.9, 127.5, 127.8, 128.2, 138.1, 180.6; CIMS *m*/*z* (%) 588 (30, $M^+ + H^+$), 569 (25), 481 (100), 434 (40); HRMS (CI^+) *m*/*z* calcd for $C_{39}H_{71}O_3$ ($M^+ + H^+$), 587.5403; found, 587.5397.

6,6′-Bis-*O*-[(2*R*,3*R*)-3-benzyloxy-2-tetradecyloctadecanoyl]-2,3,4,2′,3′,4′-hexabenzyl-α,α′-trehalose (16c) and 6-*O*-[(2*R*,3*R*)-3-Benzyloxy-2-tetradecyloctadecanoyl]-2,3,4,2′,3′,4′-hexabenzyl]-α,α′-trehalose (19c). A mixture of carboxylic acid **11c** (291 mg, 49.6 μmol), trehalose derivative **15** (292 mg, 33.0 μmol), EDCI (190 mg, 99.0 μmol), DMAP (20.2 mg, 17.0 μmol), and 4 Å molecular sieves powder in CH_2Cl_2 (4 mL) was stirred for 7 h at room temperature. The filtrate was concentrated, and the resulting material was subjected to column chromatography on silica gel using hexane and ethyl acetate (20:1 to 5:1) as the eluent to give 6,6′-bis-*O*-[(2*R*,3*R*)-3-benzyloxy-2-tetradecyloctadecanoyl]-2,3,4,2′,3′,4′-hexabenzyl-α,α′-trehalose (**16c**) (333 mg, 50%) and 6-*O*-[(2*R*,3*R*)-3-benzyloxy-2-tetradecyloctadecanoyl]-2,3,4,2′,3′,4′-hexabenzy]-α,α′-trehalose (**19c**) (201 mg, 42%) as colorless syrups. **16c**: $[\alpha]^{21}_D$ +43.5 (*c* 1.0, $CHCl_3$); FT−IR (neat) 3090, 3063, 3029, 2944, 2868, 1742 cm^{-1}; ^1H NMR (300 MHz in $CDCl_3$) δ 0.88 (12H, t, J = 6.9 Hz), 1.12−1.61 (108H, m), 2.66 (2H, ddd, J = 10.2, 6.6, and 3.2 Hz), 3.49 (2H, dd, J = 9.6 and 3.6 Hz), 3.56 (2H, t, J = 9.3 Hz), 3.62 (2H, m), 4.01 (2H, t, J = 9.6 Hz), 4.09 (2H, dd, J = 12.3 and 3.0 Hz), 4.17 (4H, m), 4.45 (4H, s), 4.51 (2H, d, J = 10.5 Hz), 4.61 (2H, d, J = 12.3 Hz), 4.67 (2H, d, J = 12.3 Hz), 4.83 (2H, d, J = 10.5 Hz), 4.84 (2H, d, J = 10.8 Hz), 4.98 (2H, d, J = 10.8 Hz), 5.10 (2H, d, J = 3.6 Hz), 7.16−7.36 (40H, m); ^{13}C NMR (50 MHz in $CDCl_3$) δ 14.1, 22.7, 24.9, 27.7, 27.8, 29.4 (2C), 29.7 (many), 31.1, 31.9, 49.8, 62.2, 69.1, 71.9, 72.9, 75.2, 75.6, 77.7, 79.6, 80.2, 81.5, 93.9, 127.4, 127.5, 127.7, 127.8, 127.9 (2C), 128.2, 128.3, 128.4 (2C), 137.8, 137.9, 138.6 (2C), 174.3; HRMS (TOF) *m*/*z* calcd for $C_{132}H_{194}O_{15}Na$ ($M^+ + Na^+$), 2042.4315; found, 2042.4277. **19c**: $[\alpha]^{19}_D$ +75.6 (*c* 1.0, $CHCl_3$); FT−IR (neat) 3501, 3088, 3063, 3031, 2925, 2853, 1947, 1870, 1807, 1736 cm^{-1}; ^1H NMR (300 MHz in $CDCl_3$) δ 0.88 (6H, t, J = 7.2 Hz), 1.12−1.64 (54H, m), 2.65 (1H, ddd, J = 10.2, 6.9, and 3.3 Hz), 3.55 (6H, m), 3.61 (1H, m), 4.04 (3H, m), 4.14 (1H, dd, J = 12.9 and 3.6 Hz), 4.21 (2H, m), 4.46 (2H, s), 4.53 (1H, d, J = 10.5 Hz), 4.65 (4H, m), 4.71 (1H, d, J = 12.0 Hz), 4.85 (2H, d, J = 11.7 Hz), 4.86 (2H, d, J = 10.8 Hz), 4.98 (1H, br d, J = 10.8 Hz), 5.08 (1H, d, J = 3.6 Hz), 5.14 (1H, d, J = 3.6 Hz), 7.17−7.38 (35H, m); ^{13}C NMR (50 MHz in $CDCl_3$) δ 14.1, 22.9, 27.7, 27.9, 29.4, 29.5, 29.7 (many), 31.1, 31.9, 49.8, 61.5, 62.2, 69.1, 71.2, 71.9, 73.0, 73.1, 75.0, 75.2, 75.6 (2C), 77.4, 77.8, 79.5, 79.7, 80.3, 81.5, 93.7, 93.9, 127.4, 127.6, 127.7, 127.9 (3C), 128.1, 128.2, 128.4 (2C), 137.8, 138.0, 138.2, 138.7 (2C), 138.8, 174.4; HRMS (TOF) *m*/*z* calcd for $C_{93}H_{126}O_{13}Na$ ($M^+ + Na^+$), 1473.9096; found, 1473.9141.

6-*O*-[(2*R*,3*R*)-3-Benzyloxy-2-tetradecyloctadecanoyl]-6′-*O*-[(2*S*,3*S*)-3-benzyloxy-2-tetradecyloctadecanoyl]-2,3,4,2′,3′,4′-hexabenzyl-α,α′-trehalose (18c). A mixture of carboxylic acid **14c** (48.0 mg, 8.20 μmol), monoester **19c** (99.0 mg, 6.80 μmol), EDCI (19.6 mg, 10.2 μmol), DMAP (4.15 mg, 3.40 μmol), and 4 Å molecular sieves powder in CH_2Cl_2 (1 mL) was stirred for 7 h at room temperature. After filtration and concentration, the residue was subjected to column chromatography on silica gel using hexane and ethyl acetate (20:1) as an eluent to give 6-*O*-[(2*R*,3*R*)-3-benzyloxy-2-tetradecyloctadecanoyl]-6′-*O*-[(2*S*,3*S*)-3-benzyloxy-2-tetradecyloctadecanoyl]-2,3,4,2′,3′,4′-hexabenzyl-α,α′-trehalose (**18c**) (121 mg, 88%) as a colorless syrup. **18c**: $[\alpha]^{19}_D$ +49.0 (*c* 1.1, $CHCl_3$); FT−IR (neat) 3088, 3064, 3031, 2929, 2855, 1946, 1870, 1805, 1739 cm^{-1}; ^1H NMR (600 MHz in $CDCl_3$) δ 0.88 (12H, t, J = 7.8 Hz), 1.12−62 (108H, m), 2.65 (1H, ddd, J = 10.8, 7.2, and 3.6 Hz), 2.69 (1H, ddd, J = 10.8, 7.2, and 3.6 Hz), 3.48 (1H, dd, J = 9.6 and 3.6 Hz), 3.51 (1H, dd, J = 9.6 and 3.6 Hz), 3.55 (1H, t, J = 9.6 Hz), 3.56 (1H, t, J = 9.6 Hz), 3.62 (2H, m), 4.00 (1H, t, J = 9.6 Hz), 4.01 (1H, t, J = 9.6 Hz), 4.08 (2H, m), 4.18

(4H, m), 4.44 (1H, d, J = 11.4 Hz), 4.45 (2H, s), 4.47 (1H, d, J = 11.4 Hz), 4.51 (1H, d, J = 10.2 Hz), 4.61 (1H, d, J = 11.4 Hz), 4.64 (1H, d, J = 12.0 Hz), 4.66 (1H, t, J = 12.0 Hz), 4.68 (1H, t, J = 12.0 Hz), 4.74 (1H, d, J = 10.8 Hz), 4.83 (4H, m), 4.97 (1H, d, J = 11.4 Hz), 4.98 (1H, d, J = 10.8 Hz), 5.08 (1H, d, J = 3.6 Hz), 5.11 (1H, d, J = 3.6 Hz), 7.13−7.36 (40H, m); ^{13}C NMR (150 MHz in $CDCl_3$) δ 14.1, 22.7, 24.9 (2C), 27.5, 27.7, 27.8 (2C), 29.4, 29.5 (2C), 29.6 (many), 29.7 (2C), 29.8 (2C), 31.0, 31.1, 31.9, 49.6, 49.8, 62.1, 62.2, 69.1, 71.8, 71.9, 72.9, 73.0, 75.2, 75.6, 75.7, 77.7, 79.5, 79.7, 80.0, 80.3, 81.5, 94.0, 127.4 (2C), 127.6, 127.7, 127.8, 127.9 (2C), 128.0, 128.2, 128.4 (4C), 137.8, 138.0 (2C), 138.6, 138.7, 174.1, 174.4; HRMS (TOF) m/z calcd for $C_{132}H_{194}O_{15}$Na (M^+ + Na), 2042.4315; found, 2042.4372.

6,6′-Bis-*O*-[(2*R*,3*R*)-3-hydroxy-2-tetradecyloctadecanoyl]-α,α′-trehalose (2c). A mixture of diester **16c** (233 mg, 11.5 μmol) and $Pd(OH)_2$ on C (16.1 mg, 2.30 μmol) in $CHCl_3$ and MeOH (1:1) (4 mL) was stirred at room temperature for 7 h under H_2 (1 atm). After filtration, the concentrated residue was subjected to column chromatography on silica gel using CH_2Cl_2/MeOH (10:1) as the eluent to give 6,6′-bis-*O*-[(2*R*,3*R*)-3-hydroxy-2-tetradecyloctadecanoyl]-α,α′-trehalose (**2c**) (141 mg, 94%) as a colorless syrup. **2c**: $[\alpha]^{24}_D$ +69.1 (*c* 1.0, $CHCl_3$); FT−IR (neat) 3385, 2928, 2855, 1730 cm^{-1}; ^{1}H NMR (600 MHz in C_5D_5N) δ 0.87 (12H, t, J = 7.1 Hz), 1.28−1.88 (106H, m), 1.98 (2H, m), 2.91 (2H, m), 4.16 (2H, t, J = 9.5 Hz), 4.21 (2H, m), 4.29 (2H, dd, J = 9.5 and 3.7 Hz), 4.69 (2H, t, J = 9.5 Hz), 4.86 (2H, dd, J = 11.7 and 5.7 Hz), 5.15 (4H, m), 5.86 (2H, d, J = 3.7 Hz); ^{13}C NMR (50 MHz in C_5D_5N) δ 14.4, 23.0, 26.4, 28.2, 29.5, 29.7 (many), 30.4, 32.2, 35.5, 54.0, 64.4, 71.6, 72.4, 72.6, 73.4, 74.8, 96.1, 175.1; FAB−MS m/z (%) 1322 (5, M^+ + Na^+), 413 (100), 329 (80), 307 (100); HRMS (FAB$^+$) m/z calcd for $C_{76}H_{146}O_{15}$Na (M^+ + Na^+), 1322.0550; found, 1322.0530.

***tert*-Butyl-9-methyl-3-oxo-8-decenoate (27).** To a stirred suspension of NaH (1.68 g, 70.2 mmol) in THF (250 mL) was dropwise added *tert*-butyl acetoacetate (**7**) (9.25 g, 58.5 mmol) at 0 °C, and the mixture was stirred for 10 min. After addition of *n*-BuLi (1.6 M hexane solution, 40.2 mL, 64.4 mmol), the mixture was stirred for an additional 30 min at the same temperature. To the resulting mixture was added 6-iodo-2-methyl-2-hexene (**26**) (19.6 g, 87.8 mmol), and it was stirred for 30 min at 0 °C. After addition of a saturated NH_4Cl solution, the organic materials were extracted with ether, and the dried and concentrated material was subjected to column chromatography on silica gel using hexane and ethyl acetate (20:1) as an eluent to give *tert*-butyl-9-methyl-3-oxo-8-decenoate (**27**) (11.0 g, 74%) as a colorless syrup. **27**: FT−IR (neat) 2978, 2931, 2858, 1739, 1717 cm^{-1}; ^{1}H NMR (300 MHz in $CDCl_3$) δ 1.35 (2H, m), 1.47 (9H, s), 1.59 (3H, br s), 1.60 (2H, m), 1.68 (3H, br s), 1.98 (2H, q, J = 7.5 Hz), 2.52 (2H, t, J = 7.2 Hz), 3.33 (2H, s), 5.09 (1H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 17.7, 23.1, 25.7, 27.7, 28.0, 28.3, 29.3, 42.9, 50.7, 81.8, 124.1, 131.7, 166.5; CIMS m/z (%) 255 (1, M^+ + H^+), 199 (40), 181 (100); HRMS (CI$^+$) m/z calcd for $C_{15}H_{27}O_3$ (M^+ + H^+), 255.1960; found, 255.1967.

(*R*)-*tert*-Butyl-3-hydroxy-9-methyl-8-decenoate (28). β-Ketoester **27** (5.00 g, 19.7 mmol) and (*R*)-BINAP−$RuCl_2$ (15.5 mg, 1.95 μmol) were dissolved in MeOH (3 mL) and deoxygenated by the freeze/melt method under an argon atmosphere. The mixture was stirred for 36 h under H_2 (50 kgf/cm^2) using a TAIATSU SUS316 microautoclave. The concentrated mixture was subjected to column chromatography on silica gel using hexane and ethyl acetate (25:2) as an eluent to give (*R*)-*tert*-butyl-3-hydroxy-9-methyl-8-decenoate (**28**) (4.10 g, 81%) as a colorless syrup. **28**: $[\alpha]^{20}_D$ −17.6 (*c* 1.4, $CHCl_3$); FT−IR (neat) 3454, 2978, 2931, 2857, 1731 cm^{-1}; ^{1}H NMR (300 MHz in $CDCl_3$) δ 1.34 (6H, m), 1.47 (9H, s), 1.60 (3H, br s), 1.68 (3H, d, J = 1.2 Hz), 1.98 (2H, m), 2.31 (1H, dd, J = 16.5 and 9.0 Hz), 2.43 (1H, dd, J = 16.5 and 3.0 Hz), 3.07 (OH, d, J = 3.9 Hz), 3.94 (1H, m), 5.10 (1H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 17.5, 25.0, 25.6, 27.8, 28.0, 29.7, 36.4, 42.3, 68.0, 81.0, 124.5, 131.2, 172.4; CIMS m/z (%) 257 (20, M^+ + H^+), 201 (100), 185 (12); HRMS (CI$^+$) m/z calcd for $C_{15}H_{29}O_3$ (M^+ + H^+), 257.2116; found, 257.2113.

(2*R*,3*R*)-*tert*-Butyl-2-icosy-3-hydroxy-9-methyl-8-decenoate (29). To a stirred solution of diisopropylamine (5.21 g, 51.5 mmol) in THF (21 mL) was added MeLi (2.2 M ether solution, 22.6 mL, 49.9 mmol) at −78 °C, and the mixture was stirred at 0 °C for 30 min. The mixture was cooled to −48 °C, and a solution of **28** (4.00 g, 15.6 mmol) in THF (10 mL) was added. After stirring for 30 min at the same temperature, HMPA (18 mL) and a solution of 1-iodoicosane (9.56 g, 23.4 mmol) in THF (14 mL) were added, and the reaction mixture was stirred for 6 h at −48 °C. After addition of saturated NH_4Cl, the organic materials were extracted with ether. The dried and concentrated extract was subjected to column chromatography on silica gel using hexane and ethyl acetate (25:1) as an eluent to give (2*R*,3*R*)-*tert*-butyl-2-icosy-3-hydroxy-9-methyl-8-decenoate (**29**) (4.60 g, 55%) as a colorless syrup. **29**: $[\alpha]^{20}_D$ +7.1 (*c* 1.3, $CHCl_3$); FT−IR (neat) 3509, 2934, 2856, 1728, 1707 cm^{-1}; ^{1}H NMR (300 MHz in $CDCl_3$) δ 0.88 (3H, t, J = 6.9 Hz), 1.10−1.41 (44H, m), 1.47 (9H, s), 1.59 (3H, br s), 1.68 (3H, d, J = 0.9 Hz), 1.98 (2H, m), 2.30 (1H, dt, J = 8.7 and 5.4 Hz), 2.65 (OH, d, J = 8.7 Hz), 3.60 (1H, m), 5.11 (1H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.1, 17.6, 22.7, 25.5, 25.7, 27.3, 27.9, 28.1, 29.3, 29.4, 29.5, 29.7 (many), 29.8, 31.9, 35.7, 51.3, 72.4, 80.9, 124.6, 131.2, 175.2; CIMS m/z (%) 537 (1, M^+ + H^+), 506 (10), 463 (100); HRMS (CI$^+$) m/z calcd for $C_{35}H_{69}O_3$ (M^+ + H^+), 537.5240; found, 537.5247.

(2*R*,3*R*)-*tert*-Butyl-3-benzyloxy-2-icosy-9-methyl-8-decenoate (30). To a solution of **29** (2.60 g, 4.84 mmol) in CH_2Cl_2 (25 mL) were added Et_3N (1.47 g, 14.5 mmol) and then TMSCl (788 mg, 7.26 mmol), and the mixture was stirred for 30 min at room temperature. After addition of brine, the organic materials were extracted with CH_2Cl_2. The dried and concentrated extract was further dried by azeotropic reflux with CH_2Cl_2 using a Soxray-type apparatus passing through a 4 Å molecular sieves column, and it was cooled to −78 °C. To the mixture were successively added benzaldehyde (770 mg, 7.26 mmol), triethylsilane (844 mg, 7.26 mol), and trimethylsilyltriflate (807 mg, 3.63 mmol). After stirring for 10 min at −78 °C, the reaction was quenched by the addition of saturated $NaHCO_3$, and the organic materials were extracted with ether. The dried and concentrated material was subjected to column chromatography on silica gel using hexane and ethyl acetate (10:1) as an eluent to give (2*R*,3*R*)-*tert*-butyl-3-benzyloxy-2-icosy-9-methyl-8-decenoate (**30**) (2.91 g, 96%) as a colorless syrup. **30**: $[\alpha]^{20}_D$ +3.5 (*c* 2.0, $CHCl_3$); FT−IR (neat) 3089, 3064, 3032, 2930, 2854, 1947, 1871, 1806, 1726 cm^{-1}; ^{1}H NMR (300 MHz in $CDCl_3$) δ 0.88 (3H, t, J = 6.9 Hz), 1.18−1.59 (44H, m), 1.43 (9H, s), 1.59 (3H, br s), 1.68 (3H, d, J = 1.2 Hz), 1.96 (2H, m), 2.55 (1H, ddd, J = 11.1, 7.5, and 3.6 Hz), 3.63 (1H, dt, J = 6.9 and 3.6 Hz), 4.50 (2H, s), 5.09 (1H, m), 7.27−7.38 (5H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.1, 17.7, 22.7, 24.3, 25.7, 27.5, 27.8, 27.9, 28.1, 29.4, 29.5 (2C), 29.6 (many), 29.7, 30.0, 30.7, 31.9, 50.3, 71.8, 80.0, 80.4, 124.7, 127.4, 127.8, 128.1, 131.2, 138.7, 174.0; CIMS m/z (%) 627 (3, M^+ + H^+), 596 (7), 571 (100); HRMS (CI$^+$) m/z calcd for $C_{42}H_{75}O_3$ (M^+ + H^+), 627.5716; found, 627.5721.

(2*R*,3*R*)-*tert*-Butyl-2-(1-benzyloxy-6-oxohexyl)docosanoate (31). Ozone gas was bubbled through a solution of **30** (1.30 g, 2.07 mmol) in $CHCl_3$ (60 mL) for 30 min at −48 °C. Excess ozone was evacuated by bubbling argon, and to this was added Me_2S (1,29 g, 20.7 mmol) at −48 °C. After warming to room temperature, saturated $NaHCO_3$ was added, and organic materials were extracted with CH_2Cl_2. The dried and concentrated material was subjected to column chromatography on silica gel using hexane and ethyl acetate (10:1) as an eluent to give (2*R*,3*R*)-*tert*-butyl-2-(1-benzyloxy-6-oxohexyl)docosanoate (**31**) (871 mg, 70%) as a colorless syrup. **31**: $[\alpha]^{19}_D$ +6.7 (*c* 1.9, $CHCl_3$); FT−IR (neat) 3089, 3064, 3032, 2927, 2853, 1728 cm^{-1}; ^{1}H NMR (300 MHz in $CDCl_3$) δ 0.88 (3H, t, J = 7.2 Hz), 1.18−1.43 (40H, m), 1.43 (9H, s), 1.56 (4H, m), 2.40 (2H, dt, J = 7.2 and 1.8 Hz), 2.55 (1H, ddd, J =

10.8, 7.2, and 3.6 Hz), 3.63 (1H, dt, J = 6.9 and 3.6 Hz), 4.47 (1H, d, J = 11.1 Hz), 4.53 (1H, d, J = 11.1 Hz), 7.28−7.34 (5H, m), 9.74 (1H, t, J = 1.8 Hz); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.0, 22.1, 22.6, 24.2, 27.5, 27.6, 28.0, 29.3, 29.4 (2C), 29.5, 29.6 (many), 30.4, 31.8, 43.7, 50.0, 71.8, 80.0, 80.1, 127.4, 127.7, 128.1, 138.5, 173.7, 202.1; CIMS *m*/*z* (%) 601 (2, $M^+ + H^+$), 584 (5), 527 (70), 437 (100); HRMS (CI^+) *m*/*z* calcd for $C_{39}H_{69}O_4$ ($M^+ + H^+$), 601.5196; found, 601.5195.

(2*R*,3*R*)-*tert*-Butyl-3-benzyloxy-2-icosyl-8-tetracosenoate (32). To a solution of hexadecanytriphenylphosphonium iodide (1.07 g, 1.74 mmol) in THF (7 mL) was added *n*-BuLi (1.6 M hexane solution, 1.00 mL, 1.60 mmol) at −78 °C, and the mixture was stirred at room temperature for 30 min and cooled to 0 °C. To this was added a solution of **31** (871 mg, 1.45 mmol) in THF (5 mL), and the mixture was stirred for an additional 30 min. After addition of saturated NH_4Cl, the organic materials were extracted with ether. The dried and concentrated material was subjected to column chromatography on silica gel using hexane and ethyl acetate (30:1) as the eluent to give (2*R*,3*R*)-*tert*-butyl-3-benzyloxy-2-icosyl-8-tetracosenoate (**32**) (1.15 g, 98%) as a colorless syrup. **32**: FT−IR (neat) 3088, 3064, 3030, 3004, 2935, 2857, 1943, 1866, 1801, 1730 cm^{-1}; ^{1}H NMR (300 MHz in $CDCl_3$) δ 0.88 (6H, t, J = 6.9 Hz), 1.18−1.62 (79H, m), 2.01 (4H, m), 2.55 (1H, ddd, J = 11.1, 7.5, and 3.6 Hz), 3.63 (1H, dt, J = 7.2 and 3.6 Hz), 4.51 (2H, s), 5.35 (2H, m), 7.21−7.36 (5H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.1, 22.7, 24.3, 27.2, 27.3, 27.5, 27.8, 28.1, 29.4, 29.5 (2C), 29.6, 29.7 (many), 29.8, 29.9, 30.7, 31.9, 50.3, 71.9, 80.1, 80.4, 127.3, 127.7, 128.1, 129.5, 130.1, 138.7, 173.9; FAB−MS *m*/*z* (%) 832 (0.1, $M^+ + H^+$), 107 (10), 91 (100); HRMS (FAB^+) *m*/*z* calcd for $C_{55}H_{100}O_3Na$ ($M^+ + Na^+$), 831.7570; found, 831.7601.

(2*R*,3*R*)-3-Benzyloxy-2-icosyl-8-tetracosenoic Acid (33). To a solution of **32** (1.00 g, 1.24 mmol) in CH_2Cl_2 (4 mL) was added trimethylsilyltriflate (55.0 mg, 24.3 μmol) at room temperature, and the mixture was stirred for 2 h at the same temperature. After addition of saturated $NaHCO_3$, organic materials were extracted with CH_2Cl_2. The dried and concentrated material was subjected to column chromatography on silica gel using hexane and ethyl acetate (10:1) as an eluent to give (2*R*,3*R*)-3-benzyloxy-2-icosyl-8-tetracosenoic acid (**33**) (831 mg, 89%) as an amorphous solid. **33**: FT−IR (neat) 3087, 3064, 3030, 3004, 2928, 2853, 1944, 1866, 1806, 1704 cm^{-1}; ^{1}H NMR (300 MHz in $CDCl_3$) δ 0.88 (6H, t, J = 6.9 Hz), 1.14−1.72 (70H, m), 2.01 (4H, m), 2.65 (1H, dt, J = 10.8 and 5.1 Hz), 3.64 (1H, q, J = 5.1 Hz), 4.52 (1H, d, J = 11.4 Hz), 4.59 (1H, d, J = 11.4 Hz), 5.37 (2H, m), 7.20−7.35 (5H, m); ^{13}C NMR (75 MHz in $CDCl_3$) δ 14.1, 22.7, 24.5, 27.1, 27.3, 27.7, 29.3, 29.4 (2C), 29.6 (2C), 29.7 (2C), 29.8 (many), 30.9, 31.0, 31.9, 32.4, 32.6, 49.7, 72.1, 79.9, 127.6, 127.8, 128.3, 129.4, 130.2, 138.1, 180.1; FAB−MS *m*/*z* (%) 776 (50, $M^+ + Na^+$), 176 (28), 91 (100); HRMS (FAB^+) *m*/*z* calcd for $C_{51}H_{92}O_3Na$ ($M^+ + Na^+$), 775.6944; found, 775.6923.

6,6′-Bis-*O*-[(2*R*,3*R*)-2-icosyl-3-hydroxytetracosanoyl]-α,α′-trehalose (2d). A mixture of diester **40** (199 mg, 8.49 μmol) and $Pd(OH)_2$ on C (11.9 mg, 1.67 μmol) in $CHCl_3$ and MeOH (3:2) (8 mL) was stirred at room temperature for 7 h under H_2 (1 atm). Filtrated and concentrated residue was subjected to column chromatography on silica gel using CH_2Cl_2 and MeOH (10:1) as the eluent to give 6,6′-bis-*O*-[(2*R*,3*R*)-2-icosyl-3-hydroxytetracosanoyl]-α,α′-trehalose (**2d**) (128 mg, 92%) as a colorless syrup. **2d**: $[\alpha]^{20}_D$ +58.5 (*c* 0.3, $CHCl_3$); FT−IR (neat) 3396, 2919, 2849, 1722 cm^{-1}; ^{1}H NMR (300 MHz in C_5D_5N) δ 0.88 (12H, t, J = 7.2 Hz), 1.14−2.06 (156H, m), 2.91 (2H, ddd, J = 10.8, 6.6, and 4.2 Hz), 4.15 (2H, t, J = 9.6 Hz), 4.21 (2H, m), 4.28 (2H, dd, J = 9.3 and 3.9 Hz), 4.69 (2H, t, J = 9.3 Hz), 4.85 (2H, dd, J = 11.7 and 5.7 Hz), 5.14 (4H, m) 5.85 (2H, d, J = 3.6 Hz); ^{13}C NMR (75 MHz in C_5D_5N) δ 14.2, 22.9, 26.3, 28.1, 29.4, 29.6, 29.8, 29.9, 30.0 (many), 32.1, 35.4, 53.8, 64.3, 71.5, 72.2, 72.5, 73.3, 74.6, 95.9, 175.1; HRMS (TOF) *m*/*z* calcd for $C_{100}H_{194}O_{15}Na$ ($M^+ + Na^+$), 1658.4315; found, 1658.4276.

Determination of the IL-6 Level on Mice Sera.[27] Briefly, a monoclonal antibody specific for mouse IL-6 was precoated onto a microplate. Recombinant IL-6 standards (ranging from 0 to 500 pg/mL), sera from TDCM-treated mice (five animals for each time), and sera from control mice (five animals for each time), corresponding to mice injected with the vehicle and maintained under the same condition as the treated mice, were pipetted into wells, and sample IL-6 was bound by the immobilized antibody. After washing to remove any unbound substances, a horseradish peroxidase-linked polyclonal antibody specific for mouse IL-6 was added to the well. Following a wash to remove any unbound antibody, a substrate solution composed of hydrogen peroxide and tetramethylbenzidine was added to the well. The enzyme reaction yielded a blue product that turned yellow when the reaction was stopped. The intensity of the color was proportional to the amount of mouse IL-6 bound in the initial step. The sample values were determined by comparison with a standard curve prepared with recombinant IL-6 (linearity assessed from 0 to 500 pg/mL; R_2 = 0.9991). For statistical evaluation, sample values were compared to the same day control values.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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