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Synthesis of macrotetrolide α, a designed polynactin analog composed of bishomononactic acids

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ABSTRACT

Macrotetrolide α (**1**), a designed polynactin analog composed of (+)- and (−)-bishomononactic acids, was synthesized. The monomeric acids were prepared using *cis*-selective iodoetherification and optical resolution of the corresponding *O*-acetylmandelates as the key steps. Esterification and macrolactonization of the monomers were performed by Corey–Mukaiyama–Gerlach method. Compound **1** showed no immunosuppressive activity contrary to other natural polynactin congeners.

1. Introduction

The macrotetrolide ionophore antibiotic 'polynactin' family (**1**), which has been isolated from various *Streptomyces* species[1,2] is composed of both enantiomers of nonactic acid (**2a**), homononactic acid (**2b**) or bishomononactic acid (**2c**) arranged in an alternating order (Fig. 1). The macrolides and monomers showed a wide variety of biological activities,[3] such as antimicrobial, fungicidal, acaricidal, immunosuppressive[4] etc., and a mixture of the parts (**1a**–**e**) is used as an acaricide by fermentative production. Their interesting structures have led many chemists to synthesize these monomers and tetramers.[5,6] Although there is no information about the biological activities of macrotetrolides B–G (**1f**–**i**),[2] the antimicrobial and antifungal activities of nonactin (**1a**), monactin (**1b**), dinactin (**1c**), and trinactin (**1d**) increase with increasing number of the ethyl substituent.[7] Accordingly, tetranactin (**1e**) and macrotetrolides B–G (**1f**–**i**) are assumed to exhibit stronger activities. Lee and Priestley reported that an imaginary analog composed of four bishomononactate units (**1j**) was calculated to show stronger K^+ binding ability,[8] which is highly correlated with biological activity. This compound **1j**, named macrotetrolide α,[9] as well as a trifluoromethyl analog, macrotetrolide β (**1k**)[10] were synthesized by

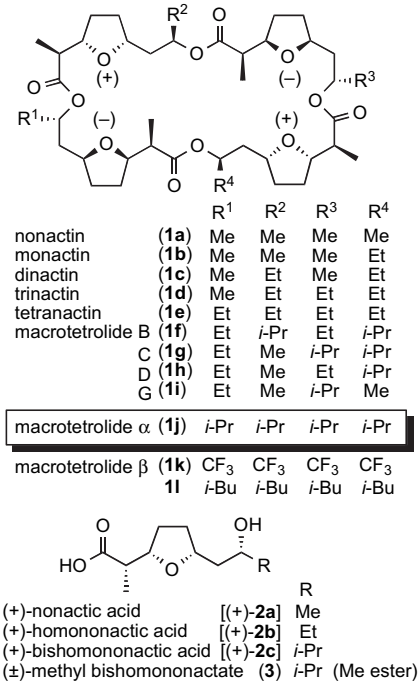

Fig. 1. Polynactin congeners and their monomers.

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us. Related analog **1l** was reported by Coutable et al.[11] Other analogs were also prepared.[12] Especially, Kusche et al. reported that nonactin analogs with all (+)- or all (−)-nonactic acids were biologically inactive.[13] In this paper, we describe the synthetic details and immunosuppressant activity of **1j**.

2. Results and discussion

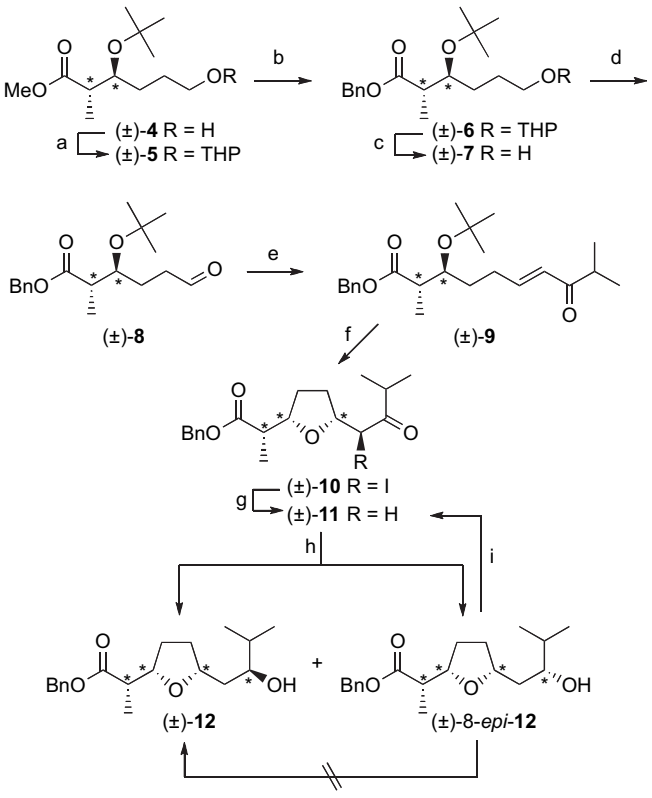
2.1. Synthetic plan

Our target macrotetrolide α (**1j**) was composed of (+)- and (−)-bishomononactic acids (**2c**) in alternating order, so **1j** could be synthesized by condensation of these monomers. We had already achieved the syntheses of (±)-**2c** (as its methyl ester)[9] and (±)- and (+)-**2b**,[14] by using cis-selective iodoetherification[15] as the key step. Since both enantiomers of **2c** were necessary to construct **1j**, resolution of (±)-**2c** or its synthetic intermediates would be effective.

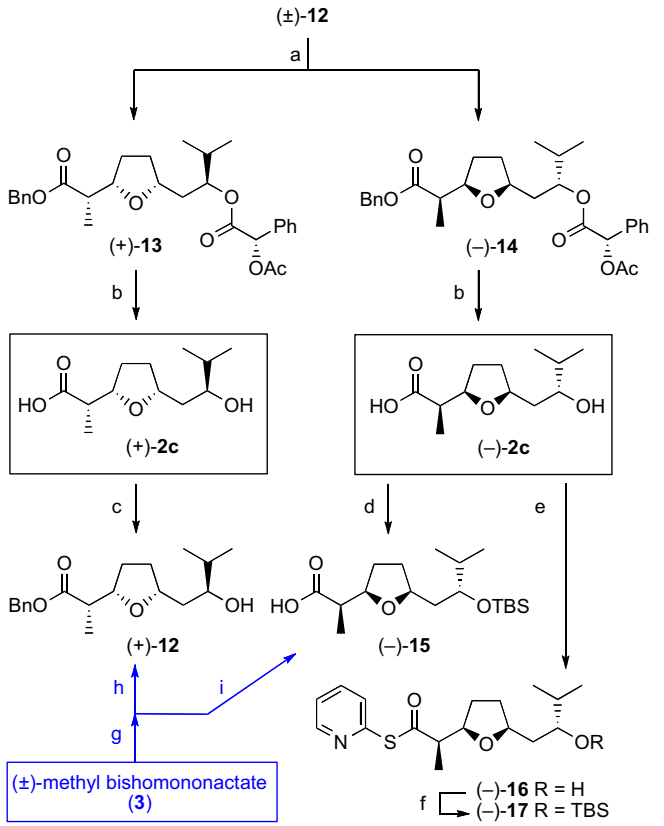
2.2. Preparation of both enantiomers of bishomononactic acid

In the previous paper, we reported a concise synthesis of (±)-methyl bishomononactate (**3**).[6b] However, the methyl ester was found to cause lowering the yield at the later hydrolysis step.[16] Thus, we prepared the monomers as benzyl esters (Scheme 1). The hydroxy group of (±)-**4**, our common synthetic intermediate for polynactins[6b] and pamamycins,[17] was protected as THP ether [(±)-**5**] that was hydrolyzed and esterified again with benzyl group to give (±)-**6**. The THP group was then removed [(±)-**7**] and the resulting hydroxy group was oxidized to afford aldehyde (±)-**8**, which was subjected to Rathke's modified Horner–Wadsworth–Emmons reaction[18] giving exclusively (*E*)-enone (±)-**9**. The iodoetherification proceeded with a complete *cis*-selectivity [(±)-**10**] and the formed iodo group was removed by treating with Bu_3SnH and AIBN to give (±)-**11**. The yield of each eight steps is above 90% (overall 71%). To resolve the racemic (±)-**11**, we first tried asymmetric reduction of the keto group of (±)-**11** [or (±)-**9**] with CBS reagent[19] or Baker's yeast.[20] However, both conditions gave products of only up to 46%ee probably due to the bulky isopropyl group, on the contrary to the superior results of the corresponding methyl ketones.[20]

Next we tried the chromatographic separation of diastereomers. Reduction of the keto group of (±)-**11** with $NaBH_4$ gave the desired (±)-**12** (40%) and (±)-8-*epi*-**12** (60%). Although a direct inversion of 8-position of the latter failed,[21] oxidation gave the parent (±)-**11** in a quantitative yield, so (±)-**12** was effectively prepared (>90% yield after five times recycle). Wang and Metz resolved (*S*)-mandelate of (±)-methyl nonactate using HPLC.[20b] We applied their method but used (*S*)-*O*-acetylmandelic acid (Scheme 2). In our case, the corresponding esters (+)-**13** and (−)-**14** were easily separated by open silica gel chromatography (R_f=0.38 for (+)-**13** and 0.43 for (−)-**14** on silica gel TLC, hexane/EtOAc=3:1), and the chiral auxiliary was quantitatively removed to give both enantiomers of bishomononactic acid (**2c**). To prepare the substrates for macrolactonization, (+)-**2c** was converted to the corresponding benzyl ester (+)-**12**. While the hydroxy group of (−)-**2c** was protected with TBS group to give (−)-**15**, or thiol ester (−)-**17** via (−)-**16**. The relative configuration of the monomers was determined by a comparison of the ^1H NMR data with those of (+)-**12** and (−)-**15** prepared from (±)-methyl bishomononactate (**3**)[9] in a similar manner. The abso-



Scheme 1. Synthesis of benzyl bishomononactate. (a) DHP, PPTS, $CHCl_3$ (96%). (b) i. 1 M aq KOH, THF–MeOH. ii. BnBr, *t*-BuOK, DMF (94%). (c) TsOH, MeOH (90%). (d) Swern oxidation. (e) $(EtO)_2P(=O)CH_2C(=O)i$-Pr, NEt_3, LiBr, THF [94% from (±)-**7**, *E* only]. (f) I_2 (6 equiv), $NaHCO_3$, CH_3CN, 20 °C, 48 h. (g) Bu_3SnH, AIBN, toluene. [93% from (±)-**9**, *cis* only]. (h) $NaBH_4$, MeOH [40% for (±)-**12** and 60% for (±)-8-*epi*-**12**]. (i) Dess–Martin periodinane (quant.).



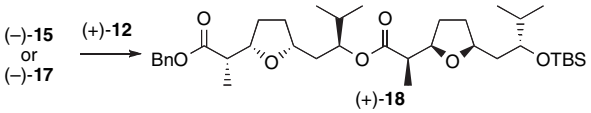
Scheme 2. Optical resolution of (±)-benzyl bishomononactate. (a) i. (*S*)-*O*-acetylmandelic acid, EDC, DMAP, CH_2Cl_2. ii. SiO_2 separation [94% for (+)-**13** and quant. for (−)-**14**]. (b) 1 M aq KOH, MeOH–THF [quant. for (+)- and (−)-**2c**]. (c) BnBr, *t*-BuOK, DMF, 65 °C (86%). (d) i. BnBr, *t*-BuOK, DMF 65 °C (84%). ii. TBSCl, Im, THF (90%). iii. H_2, 10% Pd–C, MeOH (quant.). (e) $(PyS)_2$, PPh_3, THF (74%). (f) TBSOTf, 2,6-lutidine, CH_2Cl_2 (quant.). (g) i. (*S*)-(+)-*O*-acetylmandelic acid, EDCI, DMAP, CH_2Cl_2. ii. SiO_2 separation. (h) i. 1 M aq KOH, MeOH, THF. ii. BnBr, *t*-BuOK, DMF, 65 °C (82% from **3**). (i) i. K_2CO_3, MeOH. ii. TBSCl, Im, DMF. iii. 1 M aq KOH, MeOH-THF (69% from **3**).

lute configuration was assumed by the sign of optical rotation of (+)-**2c** with those of (+)-**2a**[5d] and (+)-**2b**.[5c]

2.3. Synthesis of macrotetrolide α

As both components of the macrolide were in hand, we condensed (+)-**12** and (−)-**15**/(−)-**17** to form a dimeric compound. Table 1 shows the attempts on the condensation. Condensation with carbodiimides, such as DCC and EDC only proceeded under vigorous conditions or by adding HOBt or HOAt (entries 1–7). BOPCl gave a similar result (entry 8). Yamaguchi lactonization used by Fleming et al. in the total synthesis of nonactin (**1a**) also gave poor yields (entries 9 and 10) due to the large isopropyl substituent. Although pyridyl thiol ester (−)-**17** could be isolated by Mukaiyama–Corey method,[22] condensation did not proceed (entry 11). However, according to Gerlach et al.,[5a] addition of $AgClO_4$ promoted and completed the reaction within a few minutes to give desired (+)-**18** in 86% yield (entry 12). Removal of water from the reaction mixture by MS4 Å was also important. Unreacted compounds were recovered as (+)-**12** and (−)-**15**.

Table 1
Condensation of the monomers



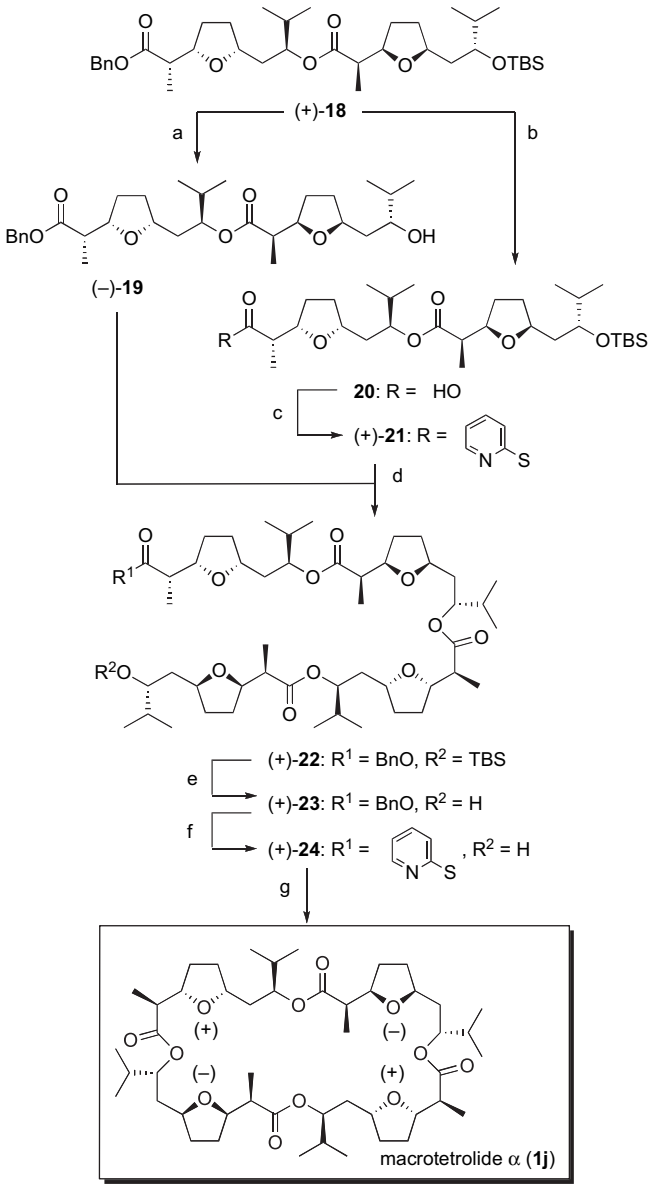
Entry	Substrate	Conditions[a]	Yield (%)
1	(−)-**15**	DCC (1.2), DMAP (0.2), rt	—
2	(−)-**15**	EDC (2), DMAP (0.2), rt	Trace
3	(−)-**15**	EDC (2), DMAP (0.2), reflux	12
4	(−)-**15**	EDC (4), DMAP (0.2), reflux	10
5	(−)-**15**	EDC (1), HOBt (1), DMAP (0.1), rt	17
6	(−)-**15**	EDC (1), HOAt (1), DMAP (0.1), rt	22
7	(−)-**15**	EDC (1), HOAt (1), DMAP (0.1), reflux	18
8	(−)-**15**	BOPCl, TEA, CH_2Cl_2	20
9	(−)-**15**	2,4,6-$Cl_3(C_6H_2)$COCl, TEA, DMAP, MS4 Å, CH_2Cl_2, rt	16
10	(−)-**15**	2,4,6-$Cl_3(C_6H_2)$COCl, DMAP, THF, toluene, 65 °C	19
11	(−)-**17**	Toluene, reflux	Trace
12	(−)-**17**	$AgClO_4$, toluene, MS4 Å, 0 °C	86

[a] Numbers in parenthesis are equivalent.

The dimer (+)-**18** was converted to the counterparts of tetramer, alcohol (−)-**19** and pyridyl thiol ester (+)-**21** via **20** (Scheme 3). Both compounds were condensed by Gerlach's method to afford acyclic tetramer (+)-**22** in 90% yield. Again the TBS and the benzyl group of (+)-**22** was successively removed in quantitative yield to give hydroxy acid (+)-**23** and then (+)-**24**. Finally, macrolactonization by using the same method gave macrotetrolide α (**1j**) in 81% yield. In this step none of octamer was detected. All the reactions were very clear and all the intermediary compounds were completely recovered in each step. So the overall yield of the condensation steps was nearly quantitative. Overall yield was 22% (42% brsm) in 25 steps from (±)-**4**.

2.4. Bioassay

Immunosuppressive activity of macrotetrolide α (**1j**) was tested by MLR (mixed lymphocyte reaction).[23] As shown in Table 2, **1j** did not show immunosuppressive (T cell proliferation prepared from mice of different in MHC antigens, BALB/c and C57BL/6) nor cytotoxic (mouse lymphoma EL4) activity compared with other polynactin congeners, such as **1c**, **1d**, and **1e**. This unexpected low activity of **1j** would be due to higher bulkiness or conformational change of the molecule with four isopropyl substituents. Difference in mechanism of action, not as an ionophore, would also be considered.



Scheme 3. Synthesis of macrotetrolide α. (a) HF, CH_3CN, 0 °C (quant.). (b) H_2, Pd–C, EtOH (quant.). (c) $(PyS)_2$, Ph_3P, toluene (98%). (d) $AgClO_4$, MS4 Å, toluene, 0 °C (90%). (e) i. HF, CH_3CN, 0 °C (quant.). (f) i. H_2, Pd–C, EtOH (88%). ii. $(PyS)_2$, Ph_3P, toluene (71%). (g) $AgClO_4$, MS4 Å, toluene, 0 °C (81%).

Table 2
Immunosuppressive and cytotoxic activities of macrotetrolide α and polynactin congeners

Compounds	IC_{50} (ng/ml)	
	MLR	EL4
Macrotetrolide α (**1j**)	63	250
Dinactin (**1c**)	3.9	7.8
Trinactin (**1d**)	3.9	7.8
Tetranactin (**1e**)	3.9	7.8
FK506	0.2	> 5
Cyclosporin A	13	> 50

3. Conclusion

Synthesis of a novel designed polynactin analog, named macrotetrolide α, was synthesized in almost quantitative yield by

assembly of (+)- and (−)-bishomononactic acids. These monomeric compounds were prepared by efficient synthesis of the corresponding racemic benzyl ester and optical resolution. Macrotetrolide α showed neither immunosuppressive nor cytotoxic activity.

4. Experimental

4.1. General

Optical rotation values were measured by a Horiba Sepa-300 polarimeter. IR spectra were recorded as films by a Jasco Report-100 spectrometer unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Inova 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C), an MR 400 (100 MHz for ^{13}C), and a Gemini 2000 (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometers in $CDCl_3$ with tetramethylsilane as an internal standard. Mass spectra were recorded with a Jeol JMS−700 spectrometer. Merck silica gel 60 (70−230 mesh) was used for column chromatography. Merck silica gel 60 F_{254} (0.50 mm thickness) was used for preparative TLC.

4.2. Methyl (2*RS*,3*RS*)-3-*tert*-butoxy-2-methyl-6-(tetrahydropyran-2′-yloxy)hexanoate [(±)-5]

To a solution of (±)-**4**[6b,17] (10 g, 43 mmol) and PPTS (1.0 g, 4.0 mmol) in dry $CHCl_3$ (200 ml) was added dropwise 3,4-dihydro-2*H*-pyran (DHP, 4.6 ml, 50 mmol) at 0 °C and the mixture was stirred at 0 °C for 5 h. Then to this was added a saturated aqueous $NaHCO_3$ solution and extracted with ether. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (5:1) gave (±)-**5** (13 g, 41 mmol, 96%) as a colorless oil. ν (film) 2960 (s), 2875 (m), 1740 (s, C=O), 1460 (m), 1360 (m) cm^{-1}; δ_H (500 MHz) 1.11 (3H, d, *J* 7.1 Hz, 2-Me), 1.19 (9H, s, *t*-Bu), 1.40−1.75 (9H, m), 1.82 (1H, m), 2.74 (1H, dq, *J* 7.1, 6.8 Hz, H-2), 3.38 (1H, m), 3.51 (1H, m), 3.67 (3H, s, OMe), 3.72 (1H, m), 3.83−3.92 (2H, m), 4.14 (1H, m), 4.58 (1H, m, OCHO); *m*/*z* (FAB, NOBA) 317 (MH^+), 233 ($MH-THP^+$); HRMS (FAB, NOBA+PEG): MH^+, found 317.2323. $C_{17}H_{33}O_5$ requires 317.2326.

4.3. Benzyl (2*RS*,3*RS*)-3-*tert*-butoxy-2-methyl-6-(tetrahydropyran-2′-yloxy)hexanoate [(±)-6]

To a solution of (±)-**5** (5.0 g, 16 mmol) in THF/MeOH (5:2, 70 ml) was added 1 M aq KOH (30 ml) at 20 °C and the mixture was stirred for 16 h. Then the reaction mixture was concentrated in vacuo; and the residue was diluted with water, acidified with 10% citric acid, and extracted with $CHCl_3$. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to give acid (4.8 g, 16 mmol, quant.) as a pale yellow oil. This was used in the next step without further purification.

To a mixture of the above mentioned acid (4.8 g, 16 mmol) and KO*t*-Bu (2.0 g, 17 mmol) in dry DMF (250 ml) was added BnBr (2.0 ml, 20 mmol) in one portion and the mixture was stirred at 65 °C for 16 h. The mixture was diluted with ether (150 ml) and washed with water. The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to give (±)-**6** (5.9 g, 15 mmol, 94%) as a pale yellow oil. ν (film) 2970 (s), 2940 (s), 2850 (m), 1740 (s, C=O), 1190 (s, C−O), 1035 (s, C−O), 755 (s), 700 (s) cm^{-1}; δ_H (500 MHz) 1.13 (3H, d, *J* 6.9 Hz, 2-Me), 1.19 (9H, s, *t*-Bu), 1.38−1.86 (10H, m), 2.79 (1H, dq, *J* 6.9, 6.6 Hz, H-2), 3.32 (1H, m), 3.50 (1H, m), 3.68 (1H, m), 3.81−3.95 (2H, m), 4.57 (1H, m, OCHO), 5.11 (1H, d, *J* 12.8 Hz, CHPh), 5.15 (1H, d, *J* 12.8 Hz, CHPh), 7.31−7.38 (5H, m, Ph); HRMS (FAB, NOBA): MNa^+, found 415.2465. $C_{23}H_{36}O_5Na$ requires 415.2461.

4.4. Benzyl (2*RS*,3*RS*)-3-*tert*-butoxy-6-hydroxy-2-methylhexanoate [(±)-7]

A mixture of (±)-**6** (5.9 g, 15 mmol) and TsOH (0.3 g, 1.5 mmol) in water/MeOH (1:1, 150 ml) was stirred at 20 °C for 15 h. Then the mixture was extracted with ether and the extract was washed with a saturated aqueous $NaHCO_3$ solution, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was chromatographed on silica gel (150 g). Elution with hexane/EtOAc (4:1) gave (±)-**7** (4.4 g, 14 mmol, 90%) as a colorless oil. ν (film) 3400 (s, O−H), 2970 (s), 2940 (s), 2870 (w), 1730 (s, C=O), 1190 (s, C−O), 1050 (s, C−O), 755 (s), 700 (s) cm^{-1}; δ_H (300 MHz) 1.12 (3H, d, *J* 7.1 Hz, 2-Me), 1.19 (9H, s, *t*-Bu), 1.40−1.75 (4H, m), 2.84 (1H, dq, *J* 7.1, 6.9 Hz, H-2), 3.53−3.62 (2H, m, H-6), 3.94 (1H, m), 5.10 (1H, d, *J* 12.6 Hz, CHPh), 5.16 (1H, d, *J* 12.6 Hz, CHPh), 7.32−7.40 (5H, m, Ph); HRMS (FAB, NOBA): MH^+, found 309.2070. $C_{18}H_{29}O_4$ requires 309.2068.

4.5. Benzyl (2*RS*,3*RS*)-3-*tert*-butoxy-2-methyl-6-oxohexanoate [(±)-8]

To a solution of oxalyl chloride (0.32 ml, 3.5 mmol) in dry THF (30 ml) was added dropwise a solution of DMSO (0.60 ml, 7.0 mmol) in dry THF (15 ml) at −78 °C, and the mixture was stirred for 5 min. Then a solution of (±)-**7** (1.0 g, 3.2 mmol) in dry THF (15 ml) was added dropwise and resulting mixture was stirred for 30 min before Et_3N (3.0 ml, 21 mmol) was added dropwise to the mixture. Then the mixture was stirred for 0.5 h while the temperature was gradually raised to 20 °C. To the reaction mixture was added water and the resulting solution was extracted with ether. The extract was washed with water, dried over anhydrous magnesium sulfate, and concentrated in vacuo to give aldehyde (±)-**8** (1.1 g, 3.2 mmol) as a pale yellow oil. This aldehyde was used in the next step without further purification. ν (film) 2720 (m), 1740 (br s, C=O), 1460 (w), 1430 (m), 1390 (m), 1360 (s), 1250 (s), 1200 (s) cm^{-1}; δ_H (300 MHz) 1.14 (3H, d, *J* 7.2 Hz, 2-Me), 1.16 (9H, s, *t*-Bu), 1.60−1.81 (2H, m, H-4), 2.42 (2H, dt, *J* 7.8, 1.6 Hz, H-5), 2.79 (1H, dq, *J* 7.1, 6.9 Hz, H-2), 3.93 (1H, m, H-3), 5.10 (1H, d, *J* 12.6 Hz, CHPh), 5.16 (1H, d, *J* 12.6 Hz, CHPh), 7.32−7.40 (5H, m, Ph), 9.69 (1H, t, *J* 1.6 Hz, CHO).

4.6. Benzyl (2*RS*,3*RS*)-3-*tert*-butoxy-2,9-dimethyl-8-oxodec-6-enoate [(±)-9]

After a mixture of $LiBr\cdot H_2O$ (0.37 g, 3.5 mmol), and dimethyl (3-methyl-2-oxobutyl)phosphonate (0.68 g, 3.5 mmol) in dry THF (15 ml) was stirred at 20 °C for 5 min, Et_3N (0.50 ml, 3.5 mmol) was added and mixture was stirred for further 10 min. Then to this was added (±)-**8** (1.1 g, 3.2 mmol), and the mixture was stirred for 6 h at 20 °C. The mixture was neutralized with a saturated aqueous NH_4Cl solution and extracted with ether. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel (150 g). Elution with hexane/EtOAc (10:1) gave (±)-**9** [1.1 g, 3.0 mmol, 94% from (±)-**7**] as a pale yellow oil. ν (film) 3050 (w, =C−H), 2970 (s), 1740 (s, C=O), 1700 (m, C=C), 1680 (s), 1635 (s), 1200 (m), 1070 (m), 710 (m) cm^{-1}; δ_H (300 MHz) 1.08−1.18 (18H, m), 1.38−1.64 (2H, m), 2.05−2.32 (2H, m), 2.75−2.85 (2H, m), 3.94 (1H, m, H-3), 5.10 (1H, d, J 12.6, CHPh), 5.17 (1H, d, J 12.6, CHPh), 6.14 (1H, dt, *J* 15.7, 1.6 Hz, H-7), 6.81 (1H, m, H-6), 7.35−7.40 (5H, m, Ph); HRMS (FAB, NOBA): MNa^+, found 397.2363. $C_{23}H_{34}O_4Na$ requires 397.2355.

4.7. Benzyl (2*RS*,3*RS*,6*SR*)-3,6-epoxy-2,9-dimethyl-8-oxodecanoate [(±)-11]

After a suspension of I_2 (1.1 g, 4.2 mmol) and $NaHCO_3$ (1.3 g, 9.4 mmol) in dry CH_3CN (15 ml) was stirred at 20 °C for 15 min

under N_2, a solution of (±)-**9** (0.50 g, 1.4 mmol) in dry CH_3CN (5 ml) was added dropwise to this mixture, and the resulting mixture was stirred at 20 °C for 24 h. Then to this was added I_2 (1.1 g, 4.2 mmol) and the mixture was stirred for additional 24 h. The reaction was quenched with a saturated aqueous $Na_2S_2O_3$ solution and brine, and extracted with ether. The extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to give iodide (±)-**10** (0.57 g, 1.3 mmol) as a pale yellow oil, which was used in the next step without further purification; δ_H (300 MHz) 1.12 (3H, d, *J* 6.9 Hz), 1.16 (3H, d, *J* 7.5 Hz), 1.70 (1H, ddt, *J* 12.0, 8.7, 6.6 Hz, H-4), 1.85 (1H, ddd, *J* 14.4, 12.3, 6.3 Hz, H-5), 1.99 (1H, dddd, *J* 12.0, 7.5, 6.3, 5.7 Hz, H-4), 2.26 (1H, ddt, *J* 13.2, 8.1, 6.9 Hz, H-5), 2.58 (1H, *J* 8.4, 7.2 Hz, H-2), 2.91 (1H, sep, *J* 6.9 Hz, H-9), 4.19 (1H, dd, *J* 8.1, 6.6 Hz, H-3), 4.23 (1H, dd, *J* 9.3, 8.4 Hz, H-6), 4.47 (1H, d, *J* 8.4 Hz, H-7), 5.09 (1H, d, J 12.6, CHPh), 5.16 (1H, d, J 12.6, CHPh), 7.30–7.40 (5H, m, Ph).

A solution of (±)-**10** (0.57 g, 1.3 mmol), Bu_3SnH (0.4 ml, 1.4 mmol), and AIBN (10 mg) in dry toluene (15 ml) was stirred at 25 °C for 2 h under N_2. Then to this was added KF, and the resulting suspension was stirred for 3 h before filtration. The filtrate was chromatographed on silica gel. Elution with hexane/EtOAc (10:1) gave (±)-**11** [0.40 g, 1.3 mmol, 93% from (±)-**9**] as a pale yellow oil. ν (film) 1730 (s, C=O), 1705 (m, C=O), 1250 (s), 1200 (s), 1155 (s), 1060 (s), 750 (m), 740 (m), 710 (s) cm^{-1}; δ_H (300 MHz) 1.07 (6H, d, *J* 6.9 Hz), 1.14 (3H, d, *J* 7.1 Hz), 1.32–1.69 (2H, m), 1.93–2.17 (2H, m), 2.44–2.65 (2H, m), 2.61 (1H, sep, *J* 7.2 Hz, H-9), 2.86 (1H, dd, *J* 8.7, 7.5 Hz, H-7), 4.06 (1H, q, *J* 6.9 Hz, H-6), 4.22 (1H, quint, *J* 6.9 Hz, H-3), 5.12 (1H, d, J 12.4, CHPh), 5.17 (1H, d, J 12.4, CHPh), 7.30–7.42 (5H, m, Ph); δ_C (125 MHz) 13.4, 17.9, 18.0, 28.5, 31.1, 41.3, 45.4, 46.3, 66.1, 75.7, 8.3, 128.0, 128.1, 128.5, 136.1, 174.5, 199.1, 213.0; HRMS (FAB, NOBA): MH^+, found 319.1911. $C_{19}H_{25}O_4$ requires 319.1911.

4.8. Benzyl (2*RS*,3*RS*,6*SR*,8*RS*)-3,6-epoxy-2,9-dimethyl-8-hydroxydecanoate [(±)-12] and its 8-epimer [(±)-8-*epi*-12]

To a solution of (±)-**11** (1.6 g, 3.6 mmol) in MeOH (150 ml) was added $NaBH_4$ (0.40 g, 9.4 mmol) at 0 °C and the mixture was stirred for 1.5 h. The reaction mixture was quenched with a saturated aqueous NH_4Cl solution and extracted with EtOAc. The extract was washed with water, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with hexane/EtOAc (7:1) gave (±)-**12** (0.46 g, 1.4 mmol, 40%) and (±)-8-*epi*-**12** (0.71 g, 2.2 mmol, 60%) as colorless oils. (±)-**12**: ν (film) 3450 (br s, O–H), 1740 (s, C=O), 700 (m) cm^{-1}; δ_H (300 MHz) 0.87 (3H, d, *J* 6.9 Hz, *gem* Me), 0.92 (3H, d, *J* 6.9 Hz, *gem* Me), 1.14 (3H, d, *J* 7.1 Hz, 2-Me), 1.57–1.72 (5H, m), 1.94–2.05 (2H, m), 2.48 (1H, d, *J* 4.6 Hz, OH), 2.60 (1H, dq, *J* 8.3, 7.1 Hz, H-2), 3.54 (1H, m, H-8), 4.04 (1H, m, H-3), 4.16 (1H, m, H-6), 5.16 (2H, s, CH_2Ph), 7.31–7.40 (5H, m, Ph) ppm; δ_C (75 MHz) 13.2, 17.7, 18.5, 28.6, 30.5, 33.5, 38.2, 45.3, 66.1, 73.5, 77.4, 80.7, 128.09, 128.12, 128.5, 136.2, 174.7; FABMS (NOBA+PEG): 321 (MH^+), 303 ($MH-H_2O^+$); HRMS (FAB, NOBA+PEG): MH^+, found 321.2068. $C_{19}H_{29}O_4$ requires 321.2064. (±)-8-*epi*-**12**: δ_H (300 MHz) 0.87 (3H, d, *J* 6.9 Hz, *gem* Me), 0.90 (3H, d, *J* 6.9 Hz, *gem* Me), 1.14 (3H, d, *J* 6.9 Hz, 2-Me), 1.4–1.75 (5H, m), 1.9–2.1 (2H, m), 2.62 (1H, dq, *J* 8.1, 7.2 Hz, H-2), 3.45 (1H, br, OH), 3.54 (1H, ddd, *J* 9.9, 5.1, 1.5 Hz, H-8), 4.04 (1H, dq, *J* 3.0, 6.9 Hz, H-6), 4.08 (1H, dt, *J* 8.1, 6.6 Hz, H-3), 5.10 (1H, d, *J* 12.6 Hz CHPh), 5.18 (1H, d, *J* 12.6 Hz, CHPh), 7.30–7.40 (5H, m, Ph).

4.9. (+)-Benzyl (2*S*,3*S*,6*R*,8*S*)-8-*O*-[(*S*)-*O*′-acetylmandeloyl] bishomononactate [(+)-13] and (−)-benzyl (2*R*,3*R*,6*S*,8*R*)-8-*O*-[(*S*)-*O*′-acetylmandeloyl]bishomononactate [(−)-14]

A mixture of (±)-**12** (235 mg, 0.733 mmol), (*S*)-*O*-acetylmandelic acid (210 mg, 1.10 mmol), EDC (210 mg, 1.10 mmol), and DMAP (13 mg, 0.1 mmol) in dry CH_2Cl_2 (20 ml) was stirred at room temperature for 2 h. To this was added a saturated aqueous $NaHCO_3$ solution and extracted EtOAc. The extract was washed with water, brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by silica gel chromatography. Elution with hexane/EtOAc (10:1) gave (−)-**14** (170 mg, 0.342 mmol, 93.3%) and (+)-**13** (181 mg, 0.364 mmol, 99.4%) as pale yellow oils. (+)-**13**: $[\alpha]_D^{24}$ +20 (*c* 0.70, $CHCl_3$). ν (KBr) 1735 (br s, C=O), 1495 (m, C=O), 1235 (s), 1060 (s), 760 (s), 700 (s) cm^{-1}; δ_H (300 MHz) 0.55 (3H, d, *J* 6.9 Hz, *gem* Me), 0.67 (3H, d, *J* 6.9 Hz, *gem* Me), 1.11 (3H, d, *J* 6.9 Hz, 2-Me), 1.47 (1H, m), 1.55–1.69 (3H, m), 1.76 (1H, ddd, *J* 14.2, 7.1, 3.9 Hz), 1.86–2.04 (2H, m), 2.20 (3H, s, COMe), 2.56 (1H, dq, *J* 7.8, 7.1 Hz, 2-H), 3.81 (1H, pseudo quint, *J* 5.8 Hz), 4.03 (1H, q, *J* 8.0 Hz), 4.92 (1H, dt, *J* 8.8, 4.4 Hz, H-8), 5.12 (1H, d, *J* 12.7 Hz, C*H*HPh), 5.16 (d, *J* 12.7 Hz, CH*H*Ph), 5.91 (1H, s, CHOAc), 7.30–7.50 (10H, m, Ph); HRMS (FAB, NOBA): MH^+, found 497.2541. $C_{29}H_{37}O_7$ requires 497.2541. (−)-**14**: $[\alpha]_D^{24}$ −4.1 (*c* 0.50, $CHCl_3$). ν (KBr) 1750 (s, C=O), 1735 (s, C=O), 1715 (s, C=O), 1495 (m, C=O), 1230 (s), 1210 (s), 1180 (s), 1050 (s), 760 (s), 700 (s) cm^{-1}; δ_H (300 MHz) 0.87 (3H, d, *J* 6.9 Hz, *gem* Me), 0.88 (3H, d, *J* 6.9 Hz, *gem* Me), 1.06 (3H, d, *J* 6.9 Hz, 2-Me), 1.36–1.56 (3H, m), 1.58–1.71 (3H, m), 1.85 (1H, dquint, *J* 4.9, 6.9 Hz), 2.20 (3H, s, Ac), 2.49 (1H, dq, *J* 7.7, 7.7 Hz, H-2), 3.23 (1H, m), 3.80 (1H, q, 7.7 Hz), 4.91 (1H, ddd, *J* 9.3, 4.3, 3.3 Hz, H-8), 5.11 (1H, d, *J* 12.4 Hz, C*H*HPh), 5.16 (d, *J* 12.4 Hz, CH*H*Ph), 5.88 (1H, s, CHOAc), 7.30–7.39 (8H, m, Ph), 7.44–7.49 (2H, m, Ph); HRMS (FAB, NOBA): MH^+, found 497.2544. $C_{29}H_{37}O_7$ requires 497.2541.

4.10. (−)-Bishomononactic acid [(−)-2c]

A solution of (−)-**14** (498 mg, 1.00 mmol) in 1 M aq KOH/MeOH/THF (3:2:4, 90 ml) was stirred at 20 °C for 5 h, and it was concentrated in vacuo. The residue was acidified with 10% aqueous citric acid and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with $CHCl_3$/MeOH/AcOH=100:1:0.5 gave (−)-**2c** (231 mg, 1.00 mmol, quant.) as a colorless oil, $[\alpha]_D^{21}$ −27 (*c* 0.080, $CHCl_3$); δ_H (300 MHz) 0.88–0.92 (3H, d, *J* 6.9 Hz, 10-H), 0.92–0.95 (3H, d, *J* 6.6 Hz, 9-Me), 1.16–1.21 (3H, d, *J* 7.1 Hz, 2-Me), 1.24–1.32 (1H, m), 1.60–1.78 (4H, m), 1.98–2.10 (2H, m), 2.46–2.56 (1H, quint, *J* 7.1 Hz, 2-H), 3.56–3.62 (1H, m, 8-H), 3.94–4.02 (1H, m, 3-H), 4.20–4.28 (1H, m, 6-H); HRMS (FAB, NOBA): MNa^+, found 253.1415. $C_{12}H_{22}O_4Na$ requires 253.1417.

4.11. (+)-Bishomononactic acid [(+)-2c]

Compound (+)-**2c** was prepared from (+)-**13** in the same manner (quant.) as a colorless oil, $[\alpha]_D^{21}$ +27 (*c* 0.090, $CHCl_3$); HRMS (FAB, NOBA): MNa^+, found 253.1415. $C_{12}H_{22}O_4Na$ requires 253.1416.

4.12. Compound (+)-12

A mixture of crude (+)-**2c** [ca. 1.00 mmol, prepared from (+)-**13**], KO*t*-Bu (560 mg, 5.0 mmol), and BnBr (0.57 ml, 0.82 g, 4.8 mmol) in DMF (30 ml) was stirred at 65 °C for 8.5 h. To the mixture was added a saturated aqueous NH_4Cl solution and ether. The organic layer was separated and washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel (hexane/EtOAc=3:1) to give (+)-**12** [276 mg, 0.861 mmol, 86% from (+)-**13**] as a pale yellow oil, $[\alpha]_D^{24}$ +3.0 (*c* 1.0, $CHCl_3$). FABMS (NOBA+PEG): 321 (MH^+), 303 ($MH-H_2O^+$); HRMS (FAB, NOBA+PEG): MH^+, found 321.2068. $C_{19}H_{29}O_4$ requires 321.2064.

4.13. (−)-*S*-2-Pyridyl (2*R*,3*R*,6*S*,8*R*)-thiobishomononactate [(−)-16]

A mixture of (−)-**2c** (54 mg, 0.23 mmol), 2,2′-dipyridyl disulfide (130 mg, 0.58 mmol), and PPh_3 (100 mg, 0.35 mmol) in dry THF (5 ml) was stirred at room temperature for 20 h and concentrated in vacuo. The residue was chromatographed on silica gel. Elution with hexane/EtOAc=3:1 gave (−)-**16** (55 mg, 0.17 mmol, 74%) as a pale yellow oil, $[\alpha]_D^{23}$ −40 (*c* 0.10, $CHCl_3$); δ_H (300 MHz) 0.88–0.98 (6H, m, H-10, 9-Me), 1.23 (3H, d, *J* 7.1 Hz, 2-Me), 1.58–1.82 (4H, m), 1.96–2.08 (2H, m), 2.6 (1H, m, OH), 2.87 (1H, q, *J* 7.2 Hz, H-2), 3.61 (1H, m, 8-H), 4.04–4.22 (2H, m, H-3, 6), 7.62–7.78 (3H, m, Ar), 8.60–8.64 (1H, m, Ar); *m*/*z* (FAB, NOBA) 438 (MH^+); HRMS (FAB, NOBA+PEG): MH^+, found 324.1638. $C_{19}H_{26}O_3NS$ requires 324.1635.

4.14. (−)-*S*-2-Pyridyl (2*R*,3*R*,6*S*,8*R*)-8-*O*-(*tert*-butyldimethylsilyl)thiobishomononactate [(−)-17]

To a solution of (−)-**16** (55 mg, 0.17 mmol) in CH_2Cl_2 (5 ml) was added 2,6-lutidine (0.29 ml, 0.26 mmol), and TBSOTf (0.29 ml, 1.3 mmol) at 0 °C and the mixture was stirred at 0 °C for 40 min. To the reaction mixture was added water and the aqueous layer was extracted with EtOAc. The combined extract was dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by preparative TLC (hexane/EtOAc=7:1) to give (−)-**17** (74 mg, 0.17 mmol, quant.) as a pale yellow oil, $[\alpha]_D^{25}$ −19 (*c* 0.96, $CHCl_3$); δ_H (300 MHz) 0.01 (3H, s, MeSi), 0.03 (3H, s, MeSi), 0.82–0.90 (15H, m, *t*-Bu, H-10, 9-Me), 1.21 (3H, d, *J* 6.9 Hz, 2-Me), 1.45–1.66 (4H, m), 1.74 (1H, m), 1.94–2.04 (2H, m), 2.83 (1H, dq, *J* 8.2, 6.6 Hz, H-2), 3.80 (1H, m), 3.94 (1H, m), 4.03 (1H, m), 7.64–7.76 (3H, m, Ar), 8.58–8.64 (1H, m, Ar); *m*/*z* (FAB, NOBA) 438 (MH^+); HRMS (FAB, NOBA+PEG): MH^+, found 438.2495. $C_{23}H_{40}O_3NSiS$ requires 438.2496.

4.15. (+)-Benzyl (2*S*,3*S*,6*R*,8*S*)-8-*O*-[(2′*R*,3′*R*,6′*S*,8′*R*)-8′-*O*′-(*tert*-buthyldimethylsilyl)bishomononactoyl]bishomononactate [(+)-18]

To a suspension of (−)-**17** (66 mg, 0.15 mmol), (+)-**12** (50 mg, 0.15 mmol), and MS4 Å (50 mg) in dry toluene (10 ml) was added $AgClO_4$ (35 mg, 0.17 mmol) at 0 °C, and the mixture was stirred for 5 min. Then to this was added a saturated aqueous $NaHCO_3$ solution, and extracted with EtOAc. The combined extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by preparative TLC (hexane/EtOAc=10: 1) to give (+)-**18** (84 mg, 0.13 mmol, 86%) as a yellow oil, $[\alpha]_D^{24}$ +7.0 (*c* 0.70, $CHCl_3$). *ν* (film) 3050 (w), 3025 (w), 2950 (s), 2875 (m), 1740 (s, C–O), 1460 (m), 1380 (m) cm^{-1}; δ_H (500 MHz) 0.02 (3H, s, MeSi), 0.03 (3H, s, MeSi), 0.80–0.82 (3H, d, *J* 6.8 Hz, *gem* Me), 0.83–0.86 (3H, d, *J* 6.8 Hz, *gem* Me), 0.88 (9H, s, *t*-Bu), 1.10 [3H, d, *J* 7.1 Hz, 2(2′)-Me], 1.12 [3H, d, *J* 7.1 Hz, 2′(2)-Me], 1.40–2.00 (14H, m), 2.50 [1H, quint, *J* 7.3 Hz, 2(2′)-H], 2.58 [1H, quint, *J* 7.3 Hz, 2′(2)-H], 3.67 (1H, dt, *J* 8.5, 3.7 Hz, 8′-H), 3.82–3.89 (2H, m, 3, 3′-H), 3.96–4.06 (2H, m, 6, 6′-H), 4.90 (1H, dt, *J* 8.6, 4.2 Hz, 8-H), 5.18 (2H, s, CH_2Ph), 7.28–7.38 (5H, m, Ph); *m*/*z* (FAB, NOBA) 669 (MNa^+),647 (MH^+); HRMS (FAB, NOBA+PEG): MH^+, found 647.4344. $C_{37}H_{63}O_7Si$ requires 647.4340.

4.16. (−)-Benzyl (2*S*,3*S*,6*R*,8*S*)-8-*O*-[(2′*R*,3′*R*,6′*S*,8′*R*)-bishomononactoyl]bishomononactate [(−)-19]

To a solution of (+)-**18** (30 mg, 0.046 mmol) in CH_3CN (5 ml) was added 40% aqueous HF in CH_3CN (5:95, 1 ml) at 0 °C and the mixture was stirred for 3 h at this temperature. Then to this was added a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The combined extract was washed with brine, dried over anhydrous magnesium sulfate and concentrated in vacuo. The residue was purified by preparative TLC (hexane/EtOAc=3:1) to give (−)-**19** (24 mg, 0.046 mmol, quant.) as a colorless oil, $[\alpha]_D^{23}$ −4.1 (*c* 0.50, $CHCl_3$). *ν* (film) 3500 (m, O–H), 3050 (w), 3025 (w), 2950 (s), 2875 (m), 1740 (s, C=O), 1460 (m), 1380 (m) cm^{-1}; δ_H (500 MHz) 0.84–0.90 (9H, m), 0.91–0.93 (3H, d, *J* 6.6 Hz), 1.10 (6H, dd, *J* 7.08, 1.46 Hz, 1, 11-Me), 1.40–2.00 (14H, m), 2.47–2.63 (2H, m), 2.62 (1H, m, OH), 3.55 (1H, m, 17-H), 3.86 (1H, m), 3.96–4.06 (2H, m), 4.10–4.17 (1H, m), 4.88–4.92 (1H, dt, *J* 8.6, 4.2 Hz, 8-H), 5.15 (2H, s, CH_2Ph), 7.28–7.38 (5H, m, Ph); *m*/*z* (FAB, NOBA) 555 (MNa^+), 533 (MH^+); HRMS (FAB, NOBA+PEG): MH^+, found 533.3479. $C_{31}H_{49}O_7$ requires 533.3476.

4.17. (+)-*S*-2-Pyridyl (2*S*,3*S*,6*R*,8*S*)-[(2′*R*,3′*R*,6′*S*,8′*R*)-8′-*O*′-*tert*-buthydimethylsilylbishomononactoyl]thiobishomononactate [(+)-21]

A suspension of (+)-**18** (30 mg, 0.046 mmol) and 10% Pd–C (cat. amount) in dry EtOH (1 ml) was stirred under hydrogen atmosphere (1 atm) at room temperature for 15 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo to give **20** (25 mg, 0.046 mmol, quant.) as a colorless oil. *ν* (film) 3200 (br s), 2950 (s), 2875 (m), 1740 (s, C=O), 1720 (m), 1460 (m), 1380 (m) cm^{-1}. This oil was used in the next step without further purification.

A solution of **20** (25 mg, 0.046 mmol), 2,2′-dipyridyl disulfide (15 mg, 0.068 mmol), and PPh_3 (29 mg, 0.068 mmol) in dry toluene (5 ml) was stirred at room temperature for 6 h and then concentrated in vacuo. The residue was purified by preparative TLC (hexane/EtOAc=4:1) to give (+)-**21** (29 mg, 0.045 mmol, 98%) as a pale yellow oil, $[\alpha]_D^{23}$ +20 (*c* 0.20, $CHCl_3$). *ν* (film) 2950 (s), 2875 (m), 1740 (s, C=O), 1710 (m), 1460 (m), 1380 (m) cm^{-1}; δ_H (500 MHz) 0.03 (6H, d, *J* 6.1 Hz, Me_2Si), 0.81–0.86 (6H, dd, *J* 15.1, 7.1 Hz), 0.87 (9H, s, *t*-Bu), 0.88–0.92 (6H, dd, *J* 9.5, 6.8 Hz), 1.11 (3H, d, *J* 7.1 Hz), 1.20 (3H, d, *J* 7.1 Hz), 1.40–2.00 (14H, m), 2.52 (1H, quint, *J* 7.1 Hz), 2.85 (1H, quint, *J* 7.1 Hz), 3.67 (1H, m, H-8′), 3.83–3.91 (2H, m), 4.01 (1H, m), 4.10 (1H, m), 4.96 (1H, dt, *J* 8.6, 4.2 Hz, 8-H), 7.28 (1H, m, Ar), 7.68–7.76 (2H, m, Ar), 8.61 (1H, m, Ar); *m*/*z* (FAB, NOBA) 672 (MNa^+), 650 (MH^+); HRMS (FAB, NOBA+PEG): MH^+, found 650.3906. $C_{35}H_{60}O_6NSiS$ requires 650.3924.

4.18. (+)-Benzyl (2*S*,3*S*,6*R*,8*S*)-8-*O*-((2′*R*,3′*R*,6′*S*,8′*R*)-8′-*O*′-{(2″*S*,3″*S*,6″*R*,8″*S*)-8″-*O*″-[(2‴*R*,3‴*R*,6‴*S*,8‴*R*)-8‴-*O*‴-*tert*-buthyldimethylsilylbishomononactoyl]bishomononactoyl}bishomononactoyl)bishomononactate [(+)-22]

To a suspension of (+)-**21** (20 mg, 0.031 mmol), (−)-**19** (21 mg, 0.040 mmol), and MS4 Å (20 mg) in dry toluene (5 ml) was added $AgClO_4$ (9.3 mg, 0.045 mmol) at 0 °C, and the resulting mixture was stirred for 5 min. To this was added a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The combined extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by preparative TLC (hexane/EtOAc=10:1) to give (+)-**22** (930 mg, 0.028 mmol, 90%) as a colorless oil, $[\alpha]_D^{22}$ +5.3 (*c* 0.91, $CHCl_3$). *ν* (film) 2950 (s), 2875 (m), 1740 (s, C=O), 1710 (m), 1460 (m), 1380 (m) cm^{-1}; δ_H (500 MHz) 0.024 (3H, s, MeSi), 0.036 (3H, s, MeSi), 0.81–0.90 (33H, m), 1.07–1.13 (12H, m), 1.40–2.00 (28H, m), 2.48–2.62 (4H, m, H-2, 11, 20, 29), 3.67 (1H, dt, *J* 8.5, 3.7 Hz, H-8‴), 3.78–3.88 (4H, m), 3.97–4.06 (4H, m), 4.86–4.92 (3H, m, H-8, 8′, 8″), 5.13 (2H, s, CH_2Ph), 7.28–7.38 (5H, m, Ph); *m*/*z* (FAB, NOBA) 1094 (MNa^+), 1072 (MH^+); HRMS (FAB, NOBA+PEG): MNa^+, found 1093.6982. $C_{61}H_{102}O_{13}SiNa$ requires 1093.6782.

4.19. (+)-Benzyl (2*S*,3*S*,6*R*,8*S*)-8-*O*-((2′*R*,3′*R*,6′*S*,8′*R*)-8′-*O*′-{(2″*S*,3″*S*,6″*R*,8″*S*)-8″-*O*″-[(2‴*R*,3‴*R*,6‴*S*,8‴*R*)-bishomononactoyl]bishomononactoyl}bishomononactoyl)bishomononactate [(+)-23]

To a solution of (+)-**22** (20 mg, 0.019 mmol) in CH_3CN (5 ml) was added 40% aqueous HF in CH_3CN (5:95, 1 ml) at 0 °C and the mixture was stirred for 3 h at this temperature. Then to this was added a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The combined extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by preparative TLC (hexane/EtOAc=3:1) to give (+)-**23** (18 mg, 0.019 mmol, quant.) as a colorless oil, $[\alpha]_D^{23}$ +11 (*c* 0.33, $CHCl_3$). *ν* (film) 3500 (w), 2950 (s), 2875 (m), 1740 (s, C=O), 1710 (m), 1460 (m), 1380 (m) cm^{-1}; δ_H (500 MHz) 0.85–0.94 (24H, m), 1.07–1.13 (12H, m), 1.40–2.00 (28H, m), 2.48–2.62 (4H, m, H-2, 2′, 2″, 2‴), 2.53 (1H, br, OH), 3.56 (1H, m, H-8‴), 3.78–3.88 (3H, m), 3.97–4.06 (4H, m), 4.15 (1H, m), 4.86–4.92 (3H, m, H-8, 8′, 8″), 5.13 (2H, m, CH_2Ph), 7.28–7.38 (5H, m, Ph); *m*/*z* (FAB, NOBA) 980 (MNa^+), 958 (MH^+); HRMS (FAB, NOBA+PEG): MNa^+, found 979.6116. $C_{55}H_{88}O_{13}Na$ requires 979.5918.

4.20. (+)-*S*-2-Pyridyl (2*S*,3*S*,6*R*,8*S*)-8-*O*-((2′*R*,3′*R*,6′*S*,8′*R*)-8′-*O*′-{(2″*S*,3″*S*,6″*R*,8″*S*)-8″-*O*″-[(2‴*R*,3‴*R*,6‴*S*,8‴*R*)-bishomononactoyl] bishomononactoyl}bishomononactoyl)thiobishomononactate [(+)-24]

A suspension of (+)-**23** (15 mg, 0.016 mmol) and 10% Pd–C (cat. amount) in dry EtOH (1 ml) was stirred under hydrogen atmosphere (1 atm) at room temperature for 15 h. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo to give acid (12 mg, 0.014 mmol, 88%) as a colorless oil. This was used in the next step without further purification.

The crude acid (12 mg, 0.014 mmol), 2,2′-dipyridyl disulfide (4.5 mg, 0.021 mmol), and PPh_3 (9 mg, 0.021 mmol) in dry toluene (5 ml) was stirred for 6 h at room temperature and concentrated in vacuo. The residue was purified by preparative TLC (hexane/EtOAc=4:1) to give (+)-**24** (9.8 mg, 0.010 mmol, 71%) as a pale yellow oil, $[\alpha]_D^{22}$ +10 (*c* 0.35, $CHCl_3$); δ_H (500 MHz) 0.86–0.94 (24H, m, 4× Me_2C), 1.06–1.14 (9H, m, 3× Me), 1.20 (3H, d, *J* 6.9 Hz, Me), 1.40–2.05 (24H, m), 2.45–2.60 (3H, m), 2.85 (1H, quint, *J* 7.7 Hz), 3.50–3.60 (2H, m), 3.765–3.93 (3H, m), 3.96–4.08 (4H, m), 4.85–5.00 (3H, m), 7.42–7.72 (3H, m), 8.60 (1H, m); HRMS (FAB, PEG): MH^+, found 960.5874. $C_{53}H_{86}O_{12}NS$ requires 960.5871.

4.21. Macrotetrolide α (1i)

To a suspension of (+)-**24** (9.0 mg, 0.0092 mmol) and MS4 Å (10 mg) in dry toluene (5 ml) was added $AgClO_4$ (2 mg, 0.01 mmol) at 0 °C, and the mixture was stirred for 30 min at 0 °C. To this was added a saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The combined extract was washed with brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo. The residue was purified by preparative TLC (hexane/EtOAc=10:1) to give **1j** (6.4 mg, 0.0075 mmol, 81%) as a colorless oil, $[\alpha]_D^{24}$ ±0 (*c* 0.02, $CHCl_3$); δ_H (500 MHz) 0.86–0.91 (24H, m), 1.07–1.13 (12H, m), 1.40–2.08 (28H, m), 2.49 (1H, dq, *J* 8.6, 6.8 Hz), 2.55–2.62 (2H, m), 2.68 (1H, quint, *J* 7.1 Hz), 3.77–3.88 (3H, m), 3.95 (1H, m), 4.00–4.08 (3H, m), 3.08–4.15 (1H, m), 4.80–4.96 (4H, m); *m*/*z* (FAB, NOBA) 872 (MNa^+), 850 (MH^+); δ_C (100 MHz, values were extracted from HMBC and HMQC spectra) 12.9, 17.3, 18.2, 28.2, 29.6, 31.6, 37.2, 46.0, 75.8, 76.6, 79.9, 173.5; HRMS (FAB, PEG): MNa^+, found 871.5554. $C_{48}H_{80}O_{12}Na$ requires 871.5547.

4.22. Bioassay

Immunosuppressive activity on T cell proliferation (prepared from mice of different in MHC antigens, BALB/c and C57BL/6) and cytotoxic activity (mouse lymphoma EL4) of samples were measured according to the literature.[23]

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