Tetrahedron Vol. 44, No. 19, pp. 6013 to 6020, 1988
Printed in Great Britain.

0040-4020/88 $3.00+.00
© 1988 Pergamon Press plc

SYNTHESIS OF TRICHOSTATIN A, A POTENT DIFFERENTIATION INDUCER OF FRIEND LEUKEMIC CELLS, AND ITS ANTIPODE†

KENJI MORI* and KOSHI KOSEKI††

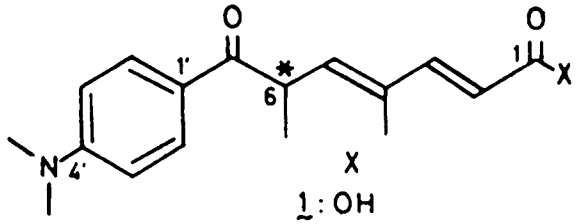
Department of Agricultural Chemistry, The University of Tokyo,

Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

(*Received in Japan* 2 *June* 1988)

Abstract — Both the enantiomers of trichostatic acid (1, 98% e.e.) and trichostatin A (2, 93% e.e.) were synthesized employing methyl (R)- or (S)-3-hydroxy-2-methylpropanoate as a starting material.

In recent years, much attention was paid to the phenomenon of differentiation of cells in connection with cancer problem. In 1976, Tsuji *et al.* isolated trichostatin A (2)[1] and C (3)[2] from metabolites of *Streptomyces hygroscopicus* as antifungal antibiotics (Fig. 1). In 1985, trichostatins were rediscovered independently by two groups[3,4,5] as very strong inducers of differentiation of Friend leukemic cells. Although Fleming *et al.*[6] synthesized racemic trichostatin A (2), the absolute configuration of trichostatins remained unknown. Recently Morioka *et al.*[7] reported that the racemic form of trichostatic acid (1) has no activity as a differentiation inducer. We became interested in the bioactivity of the enantiomers of trichostatins and also in the absolute configuration of the natural trichostatins. We therefore started our investigation to synthesize both of the enantiomers of trichostatin A (2) which has the strongest differentiation inducing potency among the trichostatins. Herein is described our results on the synthesis of the enantiomers of trichostatic acid (1) and trichostatin A (2) from a chiral building block of microbial origin.



1: OH — Trichostatic acid[2)]
2: NHOH — Trichostatin A[1)]
3: NHO-β-D-glucose — Trichostatin C[2)]

Fig.1. Structures of trichostatin A and its relatives

† Synthetic Microbial Chemistry-XX. Part XIX, T. Kitahara, A. Moriguchi and K. Mori, Tetrahedron in the press.

†† Research Fellow on leave from Japan Tobacco Inc. (1986-1988). Present Address: Life Science Research Laboratory, Japan Tobacco Inc., 6-2 Umegaoka, Midori-ku, Yokohama, Kanagawa 227, Japan

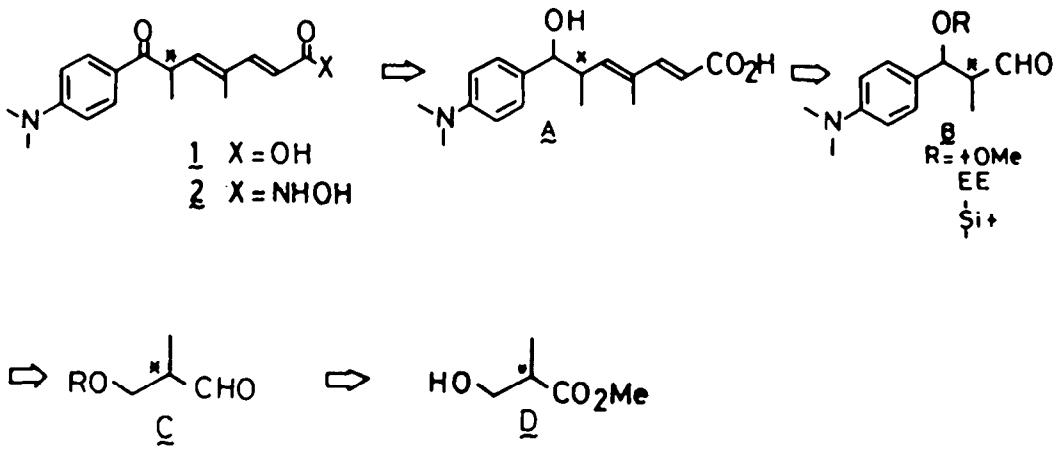


Fig. 2. Synthetic plan for trichostatin A.

Our synthetic plan is shown in Fig. 2. Our targets are both the enantiomers of trichostatin A (2) with high enantiomeric purity. Trichostatin A (2) can be derived from acid 1. To generate the chiral center activated by two carbonyl groups, the carbonyl group at C-7 postion is thought to be attached at the final stage of the synthesis so as to avoid possible racemization at C-6. Trichostatic acid (1) is therefore to be derived from the key intermediate A. A possible precursor of A is B, which is to be prepared from C. Methyl 3-hydroxy-2-methylpropanoate D is well suited for our starting material. Both the enantiomers with high e.e. of the ester D are commercially available. After several attempts, 2-methoxypropyl group was found to be an appropriate protecting group of OH at the benzylic position. As this group does not have a chiral center, analysis and purification of a reaction mixture becomes easy.

In Fig. 3 is shown our successful synthesis. The starting material, methyl (R)- or (S)-3-hydroxy-2-methylpropanoate, was converted to the corresponding (R)-α-methoxy-α-trifluoromethylphenylacetate (MTPA ester)[8]. The enantiomeric excess (e.e.) of (R)- or (S)-4 was estimated to be 99% by the HPLC analyses of their MTPA esters. After protecting the OH group of the ester (R)-4, the resulting (R)-5 was submitted to reduction with $LiBH_4$ to give alcohol (S)-6 in 82% yield. The alcohol (S)-6 was oxidized under Swern's condition[9] to give aldehyde (R)-7, which was treated with a Grignard reagent p-$Me_2NC_6H_4MgBr$[10] in THF at -40 °C to give (R)-8 in 76% yield from (S)-6. The diastereomeric ratio (syn:anti) of the resulting (R)-8 was 1:1. The diastereomers of intermediates (R)-8 to 15 could not be separated cleanly by the ordinary TLC or column chromatography, and therefore the mixtures were used without separation. The alcohol (R)-8 was treated with 2-methoxypropene in the presence of PPTS followed by $(n\text{-}Bu)_4NF$ in THF to give (R)-10 in 60% yield from (R)-8. The alcohol (R)-10 was oxidized with $DMSO/SO_3\text{-}C_5H_5N$ complex[11] to the aldehyde (R)-11, which was treated immediately with phosphorane I to give (R)-12 quantitatively from (R)-10. It should be mentioned that the oxidation of (R)-10 by Swern's method[9] produced a chlorinated substance[12].

The ester (R)-12 was reduced to the corresponding alcohol (R)-13 with DIBAL-H in toluene at -78 °C in 89% yield, and the resulting alcohol (R)-13 was oxidized in a manner similar to the oxidation of alcohol (R)-10. The resulting aldehyde (R)-14, without purification, was treated with phosphorane II to give (R)-15 in 64% yield from (R)-13. The geometry of the double bonds of the ester (R)-15 was confirmed by analyzing its 100 MHz ^1H-NMR. The $J_{Ha\text{-}Hb}$ was 15.5 Hz and n.O.e. was observed between H_a-Me_c and H_b- H_d, which suggested the diene system to be E, E. Methyl (S)-3-hydroxy-2-methylpropanoate (S)-4 was converted to (S)-16 in the same manner as described for (R)-4. We observed

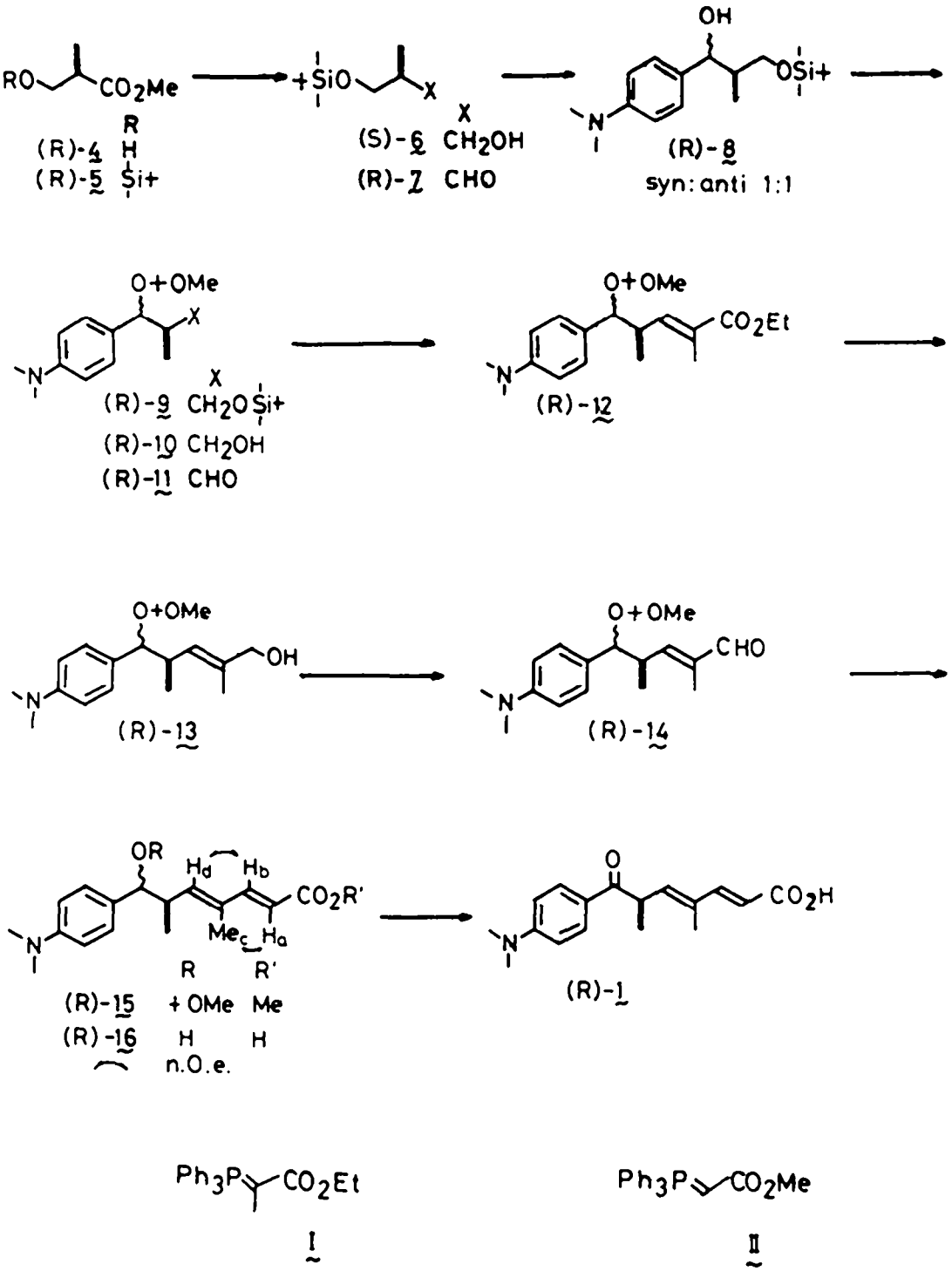


Fig. 3. Synthesis of the enantiomers of trichostatic acid

that the diastereomeric ratios of (S)-13, 14 and 15 were different from those of the corresponding (R)- intermediates. The deviation in the diastereomeric ratio might have happened because the diastereomers were separated slightly by SiO_2 column chromatography, although its TLC analysis showed a single spot. Their $[\alpha]_D$ values reflected the difference in the isomeric ratio to give values different from (R)-13, 14 and 15. The ester (R)-15 was hydrolyzed with LiOH aq. The resulting lithium salt of the acid was treated with 1N HCl at pH 2-4 to give hydroxy acid (R)-16 in 80% from (R)-15. The diastereomers of (R)-16 were separated by TLC, and an isomer could be obtained as crystals. For the sake of convenience, however, the mixture was subjected to oxidation with DDQ furnishing trichostatic acid (R)-1, m.p. 88-89 °C, $[\alpha]_D$ +138° (MeOH). (S)-Trichostatic acid (1), m.p. 88-89 °C, $[\alpha]_D$ -131° (MeOH), was obtained from (S)-12 in a similar manner as above. The naturally occurring trichostatic acid (1) was reported to show $[\alpha]_D$ +3.8° (MeOH)[2]. By comparing the sign of these optical rotations, the absolute configuration of the natural 1 was concluded to be R. The enantiomeric purities of our acids (R)- and (S)-1 were determined to be 98% by the HPLC analysis of the corresponding Me ester (CH_2N_2) using a chiral stationary phase.

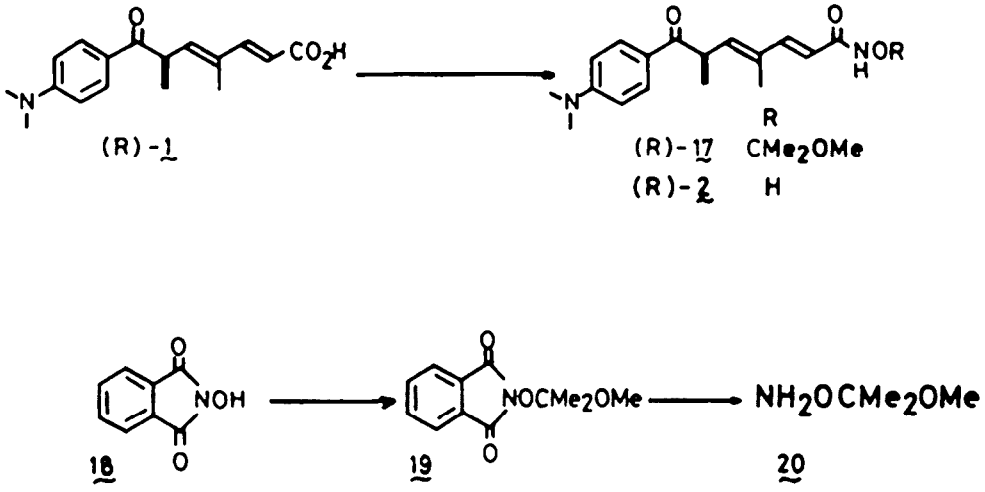


Fig. 4. Synthesis of trichostatin A

The second stage of our synthesis was the conversion of 1 to trichostatin A (2). Fleming et al.[6] treated Me ester of 1 with NH_2OH in the presence of KOH to obtain racemic 2. Tsuji et al.[2] described the condensation of NH_2OH with acyl chloride of 1 prepared from Na salt of 1 and oxalyl chloride. But these procedures were not suitable for the preparation of optically active 2. Our approach is shown in Fig. 4. Thus N-hydroxyphthalimide (18) was treated with 2-methoxypropene at room temp without catalyst for a week to give 19 and then 19 was decomposed with hydrazine to give 20, b.p. 70~73 °C/103 Torr, in 31% yield from 18. This protected hydroxylamine was to be condensed with acid (R)-1. Firstly, the activation by DCC or $(Imd)_2CO$ of the acid 1 was tried. However, all of our attempts were in vain because of the formation of N-acylated urea or C-acylated imidazole. Finally the conversion was achieved successfully by treatment with $ClCO_2Et$ in the presence of Et_3N, followed by 20 in THF at 0 °C to give (R)-17, which was deprotected immediately using Amberlyst 15 in MeOH to give trichostatin A [(R)-2], m.p. 146~150 °C (dec.), $[\alpha]_D$ +96° (MeOH), +77° (EtOH), in 27% yield from (R)-1. (S)-Trichostatin A (2), m.p. 143~149 °C, $[\alpha]_D$ -82° (MeOH), was also obtained from (S)-1. [lit. $[\alpha]_D$ +63° (MeOH)[13], +62.8° (EtOH)[1], m.p. 150~151 °C[2], 172~174 °C[13], 180~182 °C (racemic)[6]] The enantiomeric purities of (S)- and (R)-2 were determined by the HPLC

analysis as follows. As the hydroxamic acid 2 gave only poor-shaped elution pattern, it was methylated with CH_2N_2. The dimethylated 2 was separated by HPLC using a chiral stationary phase column. Although the presence of (S)-isomer in the sample of (R)-2 was not detected because of tailing of the (R) peak, (R)-isomer contaminated in (S)-2 could be separated. The enantiomeric excess of (S)-2 was calculated as >93%. That of (R)-2 was estimated to be >93% by comparison of $[\alpha]_D$ values with that of (S)-2. The absolute configuration of the natural trichostatin A (2) was determined to be R, as its CD curve agreed with that of (R)-2.

In conclusion, we completed a chiral synthesis of both the enantiomers of trichostatic acid (1) and trichostatin A (2) with high enantiomeric purities (98 and 93%). The absolute configuration of naturally occurring trichostatic acid (1) and that of trichostatin A (2) were determined to be R. The biological study on our enantiomers of 1 and 2 is now underway in Prof. T. Beppu's Laboratory of our Department. Finally it is worthy of note that Ōmura and his co-workers recently discovered antitrichomonal activity of trichostatin A (2)[14].

EXPERIMENTAL

All bps and mps were uncorrected. IR spectra were measured as films for oils or KBr disks for solids on a Jasco IRA-102 spectrometer. ^{1}H-NMR spectra were recorded with TMS as an internal standard at 100 MHz on a Jeol JNM FX-100 spectrometer. ^{13}C-NMR spectra were measured on the same FX-100 spectrometer at 25 MHz. Optical rotations were measured on a Jasco DIP-140 polarimeter. CD spectra were recorded on a Jasco J-20 automatic spectropolarimeter. UV spectra were measured on a Shimadzu UV-160 .

Methyl 3-t-butyldimethylsilyloxy-2-methylpropanoate 5

(a) (R)-Isomer. To a stirred soln of methyl (R)-2-methyl-3-hydroxypropanoate (10 g, 84.7 mmol)($[\alpha]_D^{25}$ -26.1° (c=3.43, MeOH 99% e.e.) and imidazole (12.7 g, 185 mmol) in DMF (100 ml) was added t-butyldimethylsilyl chloride (14 g, 93 mmol). The reaction mixture was stirred overnight at room temperature. It was then poured into ice-water and extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled to give 19.0 g (96.5%) of (R)-5 as a colorless oil, b.p. 108-110 °C/24 Torr, n_D^{19} 1.4205; $[\alpha]_D^{24}$ -18.4° (c=2.02, $CHCl_3$); νmax(film) 2950 (s), 2850 (s), 1740 (s), 1090 (s) cm^{-1}; δ ($CDCl_3$) 0.08 (6H, s), 0.90 (9H, s), 1.15 (3H, d, J=6.9 Hz), 2.67 (1H, heptet, J=6.4 Hz), 3.07 (3H, s), 3.65 (1H, dd, J=6.4 and 9.3 Hz), 3.75 (1H, dd, J=6.4 and 9.3 Hz). (Found: C, 56.63; H, 10.32. Calc for $C_{11}H_{24}O_3Si$: C, 56.85; H, 10.41%).

(b) (S)-Isomer. In the same manner as described above, (S)-4 (5.29 g, 44.8 mmol) gave 9.50 g (91.3%) of (S)-5 whose spectral data were identical with those of (R)-5, n_D^{19} 1.4201; $[\alpha]_D^{24}$ +18.8° (c=2.06, $CHCl_3$). (Found: C, 56.54; H, 10.43. Calc for $C_{11}H_{24}O_3Si$: C, 56.85; H, 10.41%).

2-Methyl-3-t-butyldimethylsilyloxy-1-propanol 6

(a) (S)-Isomer. To an ice-cooled suspension of $LiBH_4$ (0.9 g, 41.3 mmol) in THF (50 ml), a soln of (R)-5 (15.4 g, 66.2 mmol) in THF (80 ml) was added over 30 min, and the mixture was stirred under reflux for 5 h. Then a soln of sat NH_4Cl (15 ml) was added to the ice-cooled reaction mixture and the mixture was extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled to give 11.1 g (81.7%) of (S)-6 as a colorless oil, b.p. 105-107 °C/17 Torr; n_D^{23} 1.4274; $[\alpha]_D^{23}$ -10.8° (c=1.19, $CHCl_3$); νmax 3380 (br), 2950 (s), 2850 (s), 1250 (s), 1090 (s), 1035 (s) cm^{-1}; δ ($CDCl_3$) 0.12 (6H, s), 0.88 (3H, d, J=6.9 Hz), 0.94 (9H, s), 1.94 (1H, m), 2.25(1H, br. OH), 3.70 (4H, m), (Found: C, 58.55; H, 11.73. Calc for $C_{10}H_{24}O_2Si$: C, 58.77; H, 11.84%).

(b) (R)-Isomer. In the same manner as described above, (S)-5 (5.0 g, 21.5 mmol) gave 3.40 g (77.1%) of (R)-6 whose spectral data were identical with those of (S)-6, n_D^{19} 1.4289; $[\alpha]_D^{24}$ +10.9° (c=2.13, $CHCl_3$). (Found: C, 58.45; H, 11.81. Calc for $C_{10}H_{24}O_2Si$: C, 58.77; H, 11.84%).

3-t-Butyldimethylsilyloxy-1-(4'-N,N-dimethylaminophenyl)-2-methyl-1-propanol 8

(a) (2R)-Isomer. To a soln of oxalyl chloride (5.37 g, 42.3 mmol) in dry CH_2Cl_2 (40 ml) was added dropwise a soln of dry DMSO (5.30 g, 67.8 mmol) in CH_2Cl_2 (20 ml) at -78 °C under Ar, and the mixture was stirred for 15 min at -78 °C. Then alcohol (S)-6 (6.2 g, 30 mmol) in dry CH_2Cl_2 (20 ml) was added dropwise to the mixture at -60—70 °C. After 15 min, Et$_3$N (13.7 g, 135 mmol) was added to the mixture and then the reaction temp was raised to 0 °C over 30 min. The mixture was stirred at 0 °C for 15 min and then warmed to room temp. To this was added iced-water and the mixture was extracted with ether. The ether soln was washed with water, brine, dried ($MgSO_4$) and concentrated in vacuo below 40 °C. This was used immediately without further purification. To a soln of a Grignard reagent prepared from p-bromo-N,N-dimethylaniline (11.4 g, 56.8 mmol) and Mg (2.8 g, 118 mmol) in dry THF (60 ml) was added a soln of the aldehyde (R)-7 in THF (40 ml) at -35 °C with vigorous stirring. After 10 min sat NH_4Cl soln was added and the reaction temp was raised to room temp. The reaction mixture was diluted with water and extracted with ether. The ether soln was washed with water and brine, dried

($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 to give 7.48 g (76.2%) of (R)-8 as a yellow oil. n_D^{19} 1.5072; $[\alpha]_D^{24}$ -3.0° (c=1.14, $CHCl_3$); νmax 3450 (br, m), 2950 and 2860 (s), 2800 (m), 1610 (s), 1520 (s), 1080 (s), 1020 (m), 840 (s) cm^{-1}; δ ($CDCl_3$) 0.10 and 0.12 (total 6H, both s), 0.72 and 0.85 (total 3H, both d, J=7.0 Hz), 0.94 (9H, s), 1.80~2.20 (1H, m), 2.96 (6H, s), 3.50~3.90 (2H), 4.46 (ca. 0.5H, d, J=7.7 Hz), 4.84 (ca. 0.5H, d, J=3.7 Hz), 6.72 (2H, d, J=9.3 Hz), 7.20(2H, d, J=9.3 Hz); (Found: C, 66.79; H, 10.31; N, 4.36. Calc for $C_{18}H_{33}O_2NSi$: C, 66.82; H, 10.28; N, 4.33%).

(b) (2S)-Isomer. In the same manner as described above, (R)-6 (10 g, 49 mmol) gave 12 g (76%) of (S)-8 whose spectral data were identical with those of (R)-8, n_D^{19} 1.5059; $[\alpha]_D^{24}$ +5.79° (c=1.24, $CHCl_3$). (Found: C, 67.05; H, 10.25; N, 4.45. Calc for $C_{18}H_{33}O_2NSi$: C, 66.82; H, 10.28; N, 4.33%).

3-t-Butyldimethylsilyloxy-1-(4'-N,N-dimethylaminophenyl)-1-(2-methoxypropyloxy)-2-methylpropane 9

(a) (2R)-Isomer. To a soln of alcohol (R)-8 (6.8 g, 21 mmol) in 2-methoxypropene (10 ml) was added PPTS (0.3 g) and the mixture was stirred for 3 h at room temp. To this was added sat $NaHCO_3$ soln and the mixture was extracted with ether. The ether soln was washed with water, brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 to give 6.03 g (78.5%) of (R)-9 as an oil, n_D^{23} 1.4863; $[\alpha]_D^{22}$ -6.05° (c=1.56, $CHCl_3$); νmax 2950 (s), 2870 (s), 1620 (s), 1520 (s), 1070 (s), 1030 (s), 840 (s) cm^{-1}; δ ($CDCl_3$) 0.00 and 0.06 (total 6H, both s), 0.67 and 1.00 (total 3H, d, J=6.4 Hz), 0.90 and 0.95 (total 9H, both s), 1.15 and 1.36 (total 6H, both s), 1.7~2.3 (1H), 2.95 (6H, s), 3.02 (ca. 1.5H, s), 3.05 (ca. 1.5H, s), 3.18(ca. 0.5H, dd, J=6.5 and 10 Hz), 3.48 (ca. 1H, d, J=6.2 Hz), 3.55 (ca. 0.5H, dd, J=6.7 and 10 Hz), 4.58 (ca. 0.5H, d, J=6.7 Hz), 4.69 (ca. 0.5H, d, J=6.7 Hz), 6.69 (2H, d, J=8.7 Hz), 7.15 (2H, d, J= 8.7 Hz); (Found: C, 66.87; H, 10.30; N, 3.67. Calc for $C_{22}H_{41}O_3NSi$: C, 66.79; H, 10.44; N, 3.54%).

(b) (2S)-Isomer. In the same manner as described above, (S)-8 (7.0 g, 21 mmol) gave 7.8 g (90%) of (S)-9, whose spectral data were identical with those of (R)-9, n_D^{19} 1.4866 ; $[\alpha]_D^{23}$ +6.25° (c=1.38, $CHCl_3$). (Found: C, 67.04; H, 10.71; N, 3.69. Calc for $C_{22}H_{41}O_3NSi$: C, 66.79; H, 10.44; N, 3.54%).

3-(4'-N,N-dimethylaminophenyl)-3-(2-methoxypropyloxy)-2-methyl-1-propanol 10

(a) (2R)-Isomer. To a soln of (R)-9 (5.23 g, 13.2 mmol) in dry THF (30 ml) was added $(n\text{-}Bu)_4NF$ in THF (1 M soln, 15 ml) and the mixture was stirred for 4 h at 40 °C. To this was added sat NH_4Cl soln and the mixture was extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 to give 3.1 g (83.5%) of (R)-10 as an oil, n_D^{19} 1.5250; $[\alpha]_D^{24}$ -8.71° (c=1.40, $CHCl_3$); νmax 3450 (br), 2950 (s), 1620 (s), 1520 (s), 1070 (s), 1030 (s) cm^{-1}; δ ($CDCl_3$) 0.68 and 0.72 (total 3H, each d, J=6.7 Hz), 1.12 and 1.40(total 6H, each s), 1.85~2.30 (1H), 2.95 (6H, s), 3.10 (ca. 1.5H, s), 3.18 (ca. 1.5H, s), 3.25~3.90 (2H+OH), 4.47 (ca. 0.5H, d, J=6.0 Hz), 4.80 (ca. 0.5H, d, J=4.3 Hz), 6.68 (2H, d, J=8.7 Hz), 7.16 (2H, d, J=8.7 Hz); (Found: C, 67.82; H, 9.60; N, 4.99. Calc for $C_{16}H_{27}O_3N$: C, 68.29; H, 9.67; N, 4.98%).

(b) (2S)-Isomer. In the same manner as described above, (S)-8 (6.0 g, 15.2 mmol) gave 3.4 g (80%) of (S)-9 whose spectral data were identical with those of (R)-9, n_D^{19} 1.5244 ; $[\alpha]_D^{24}$ +7.56 (c=2.05, $CHCl_3$). (Found: C, 68.05; H, 9.54; N, 5.08. Calc for $C_{16}H_{27}O_3N$: C, 68.29; H, 9.67; N, 4.98%).

Ethyl 2,4-dimethyl-5-(4'-N,N-dimethylaminophenyl)-5-(2-methoxypropyloxy)-2-pentenoate 12

(a) (4R)-Isomer. To a soln of alcohol (R)-10 (2.38 g, 8.45 mmol) in dry DMSO (21.6 ml) and Et_3N (7.5 ml) was added SO_3-C_5H_5N complex (3.98 g, 25 mmol) in DMSO (21.6 ml). The mixture was stirred at room temp for 5 min. To this was added iced-water and this was extracted with ether. The ether soln was washed with water and brine, dried ($MgSO_4$) and concentrated in vacuo below 40 °C. This (R)-11 was subjected to the next reaction without further purification. To a soln of (R)-11 in dry CH_2Cl_2 (23 ml) was added ethyl 2-(triphenylphosphoranylidene)propionate (7.6 g, 21 mmol) in one portion and the mixture was stirred under gentle reflux for 6 h under Ar. The mixture was then concentrated in vacuo. To it was added 50 ml of 10% EtOAc in hexane and the mixture was filtered. The precipitate was washed thoroughly with the same solvent. The filtrate was concentrated in vacuo, and the residue was chromatographed over SiO_2 to give 3.4 g (quantitative) of (R)-12 as an oil, n_D^{22} 1.5119; $[\alpha]_D^{24}$ +31.3° (c=2.39, $CHCl_3$); νmax 3000 (s), 1710 (s), 1620 (m), 1520 (s), 1080 (m), 1030 (s), 750 (m) cm^{-1}; δ ($CDCl_3$) 0.79 and 1.02 (total 3H, each d, J=7.1 Hz), 1.07 and 1.10 (total 3H, each s), 1.26 and 1.29 (total 3H, each t, J=7.1 Hz), 1.30 and 1.38 (total 3H, each s), 1.78 and 1.88 (total 3H, each d, J=1.9 Hz), 2.60~2.95 (1H), 2.94 and 2.96 (total 6H, each s), 3.06 and 3.12 (total 3H, each s), 4.00~4.32 (2H), 4.42 (ca. 0.5H, d, J=8.0 Hz), 4.51 (ca. 0.5H, d, J=6.0 Hz), 6.50~6.73 (1H), 6.65 (ca. 1H, d, J=9.3 Hz), 6.68 (ca. 1H, d, J=9.3 Hz), 7.12 (ca. 1H, d, J=9.3 Hz), 7.15 (ca. 1H, d, J=9.3 Hz). (Found: C, 69.41; H, 9.06; N, 3.88. Calc for $C_{21}H_{33}O_4N$: C, 69.39; H, 9.15; N, 3.85%).

(b) (4S)-Isomer. In the same manner as described above, (S)-10 (4.0 g, 14.2 mmol) gave 3.06 g (59%) of (S)-12 whose spectral data were identical with those of (R)-12, n_D^{19} 1.5112; $[\alpha]_D^{26}$ -33.5° (c=2.38, $CHCl_3$). (Found: C, 69.57; H, 8.79; N, 3.85. Calc for $C_{21}H_{33}O_4N$: C, 69.39; H, 9.15; N, 3.85%).

2,4-Dimethyl-5-(4'-N,N-dimethylaminophenyl)-5-(2-methoxypropyloxy)-2-penten-1-ol 13

(a) (4R)-Isomer. To a soln of ester (R)-12 (3.05 g, 8.39 mmol) in dry toluene (38 ml) was added dropwise DIBAL-H (1 M in n-hexane, 20 ml) at -55 °C~-60 °C under Ar. The mixture was stirred for 30 min at -78 °C. Then, to this was added sat Rochelle salt soln (20 ml) and the temp of the mixture was raised to room temp. The mixture was filtered through a Celite pad and the pad with the precipitate was washed thoroughly with toluene. The combined toluene soln was washed with sat Rochelle salt soln, dried ($MgSO_4$) and concentrated in vacuo. The residue was chromatographed over SiO_2 to give 2.4 g (89%) of alcohol (R)-13 as a very viscous oil, n_D^{19} 1.5558; $[\alpha]_D^{24}$ -10.6° (c=2.32, $CHCl_3$); νmax 3400 (br), 2950 (s), 1620 (s), 1520 (s), 1070 (m), 1020 (s) cm^{-1}; δ ($CDCl_3$) 0.80 and 1.00 (total 3H, d, J= 6.7 Hz), 1.08, 1.10, 1.32 and 1.38(total 6H, each s), 1.52 and 1.65 (total 3H, d, J=1.6 Hz), 2.60~3.00 (1H), 2.93 and 2.96 (total 6H, each s), 3.08 and 3.10 (total 3H, each s), 3.50 (1H,OH), 3.88 and 3.99 (total 2H, d, J=5.2 Hz), 4.40 and 4.42(total 1H, d, J=6.5 Hz), 5.10 (ca. 0.5H, d, J=7.5 Hz), 5.19 (ca. 0.5H, d, J=6.5 Hz), 6.63 and 6.67 (total 2H, d, J=8.6 Hz), 7.10 and 7.12 (total 2H, d, J=8.6 Hz). (Found: C, 71.20; H, 9.57; N, 4.26. Calc for $C_{19}H_{31}O_3N$: C, 70.99; H, 9.72; N, 4.36%).

(b) (4S)-Isomer. In the same manner as described above, (S)-12 (3.05 g, 8.39 mmol) gave 2.04 g (76%) of (S)-13 whose spectral data were identical with those of (R)-13, n_D^{19} 1.5528; $[\alpha]_D^{21}$ +34.1° (c=1.30, $CHCl_3$). (Found: C, 70.88; H, 9.56; N, 4.36%. Calc for $C_{19}H_{31}O_3N$: C, 70.99; H, 9.72; N, 4.36%).

Methyl 4,6-dimethyl-7-(4'-N,N-dimethylaminophenyl)-7-(2-methoxypropyloxy)-2,4-heptadienoate 15

(a) (6R)-Isomer. The alcohol (R)-13 (1.86 g, 5.8 mmol) was oxidized to the corresponding aldehyde (R)-14 in the same manner as described for the oxidation of (R)-10 using SO_3-C_5H_5N complex (2.72 g, 17.1 mmol), DMSO (30 ml) and Et_3N (7.5 ml). The

resulting aldehyde acid was employed in the next reaction without purification. To a soln of (R)-14 in dry CH_2Cl_2 (30 ml) was added methyl triphenylphosphoranylideneacetate (4 g, 12 mmol) and the mixture was stirred under gentle reflux for 24 h under Ar. Conventional work-up as described in the case of (R)-12 gave 1.4 g (64%) of (R)-15 as an oil, n_D^{19} 1.5364; $[\alpha]_D^{23}$ -6.76° (c=1.77, $CHCl_3$); νmax 3000 (m), 2880 (br), 1720 (s), 1630 (sh), 1520 (s), 1170 (m), 1070 (s), 1020 (s) cm^{-1}; δ ($CDCl_3$) 0.84 and 1.01 (total 3H, d, J=6.6 Hz), 1.08, 1.10, 1.32 and 1.37 (total 6H, each s), 1.89 and 1.78 (total 3H, d, J=1.3 Hz), 2.7-2.9 (1H), 2.94 and 2.96 (total 6H, each s), 3.05 and 3.08 (total 3H, each s), 3.74 and 3.76 (total 3H, each s), 4.43 (ca. 0.5H, d, J=6.5 Hz), 4.68 (ca. 0.5H, d, J=6.0 Hz), 5.60 and 5.72 (total 1H, bd, J=10.0 Hz), 5.74 and 5.80 (total 1H, d, J=15.0 Hz), 6.62 and 6.65 (total 2H, d, J=9.0 Hz), 7.07 and 7.10 (total 2H, d, J=9.0 Hz), 7.25 and 7.32 (total 1H, d, J=15.0 Hz). (Found: C, 70.18; H, 9.02; N, 3.64. Calc for $C_{22}H_{33}O_4N$: C, 70.37; H, 8.86; N, 3.73%).

(b) (6S)-Isomer. In the same manner as described above, (S)-13 (0.89 g, 2.71 mmol) gave 0.82 g (81%) of crude (S)-15, whose spectral data were virtually identical with those of (R)-15, n_D^{19} 1.5392; $[\alpha]_D^{18}$ +14.5° (c=1.95, $CHCl_3$). (Found: C, 70.05; H, 8.82; N, 3.80. Calc for $C_{22}H_{33}O_4N$: C, 70.37; H, 8.86; N, 3.73%).

2,4-Dimethyl-7-(4'-N,N-dimethylaminophenyl)-7-hydroxy-2,4-heptadienoic acid 16

(a) (6R)-Isomer. To a soln of ester (R)-15 (1.4 g, 3.73 mmol) in methanol (29.5 ml) was added aq LiOH soln (0.52 M, 9.85 ml, 5.12 mmol), and the mixture was stirred for 12 h at 45 °C. After adjusting the pH of the mixture to 7-8 using 1N HCl, it was concentrated in vacuo. The residue was acidified to pH 3-4 with 1N HCl, and the mixture was stirred for 10 min at room temp. It was then extracted with $CHCl_3$-MeOH (95:5). The organic layer was washed with brine, dried ($MgSO_4$) and concentrated in vacuo to give 0.86 g (80%) of acid (R)-16 as a yellow amorphous solid. The acid was used for the next step without purification. A small portion of it was treated with $CHCl_3$-EtOH (2:1) to give an analytical sample of a single diastereomer of the acid as a yellow amorphous solid, m.p. 165-167 °C; $[\alpha]_D^{22}$ +156° (c=0.50, 10% MeOH/ $CHCl_3$); νmax 3300 (br), 2900 (br), 1660 (s), 1620 (m), 1530 (m), 1410 (m) cm^{-1}; δ (CD_3OD+$CDCl_3$+1:9) 0.89 (3H, d, J=6.5 Hz), 1.80 (3H, d, J=1.0 Hz), 2.6-3.0 (1H), 2.97 (6H, s), 4.40 (1H, d, J=7.0 Hz), 5.80 (1H, d, J= 15.5 Hz), 5.89 (1H, bd, J=9.5 Hz), 6.74 (2H, d, J=8.5 Hz), 7.20 (2H, d, J=8.5 Hz), 7.40 (1H, d, J=15.5 Hz). (Found: C, 70.19; H, 7.79; N, 4.78. Calc for $C_{17}H_{23}O_3N$: C, 70.56; H, 8.01; N, 4.84%).

(b) (6S)-Isomer. In the same manner as described above, (S)-15 (0.75 g, 2.0 mmol) gave 0.58 g (quantitative) of (S)-16, whose spectral data were identical with those of (R)-16, m.p. 156-159 °C; $[\alpha]_D^{19}$ -132° (c=0.50, 10% MeOH/ $CHCl_3$). (Found: C, 70.01; H, 7.99; N, 4.82. Calc for $C_{17}H_{23}O_3N$: C, 70.56; H, 8.01; N, 4.84%).

Trichostatic acid 1

(a) (R)-Isomer. To a soln of (R)-16 (325 mg, 1.3 mmol) in dry dioxane (5 ml) was added DDQ (300 mg, 1.3 mmol) in dry dioxane (3 ml) and the mixture was stirred for 5 min at room temp under Ar. It was filtered and the precipitate was washed with dioxane. The filtrate was concentrated in vacuo and the residue was chromatographed over SiO_2 (benzene : 2-propanol 98.5 :1.5 ~ 96:4) to give 112 mg (34%) of crude (R)-trichostatic acid (R)-1. Recrystallization from ether gave a pure sample as slightly yellow plates, m.p. 88-89 °C (lit. 138-140 °C[2]); $[\alpha]_D^{20}$ +138° (c=0.35, MeOH) (lit. +1.8° (c=1.033 MeOH)[2]); ν max 2950 (br), 1680 (m), 1600 (s), 1370 (m) cm^{-1}; δ ($CDCl_3$) 1.31(3H, d, J=6.8 Hz), 1.92 (3H, br s), 3.07 (6H, s), 4.40 (1H, dq, J=9.6 and 6.8 Hz), 5.81(1H, d, J=15.6 Hz), 6.09 (1H, br d, J=9.6 Hz), 6.64 (2H, d, J=8.8 Hz), 7.38 (1H, d, J=15.6 Hz), 7.84 (2H, d, J=8.8 Hz); ^{13}C-NMR δ ($CDCl_3$+77.0 ppm) 12.5, 17.7, 40.0, 40.8, 110.8, 115.8, 123.9, 130.6, 132.6, 142.9, 151.3, 153.5, 172.2, 198.3. (Found: C, 70.72; H, 7.50; N, 4.71. Calc for $C_{17}H_{21}O_3N$: C, 71.06; H, 7.37; N, 4.87%). The IR and NMR spectra were identical with the reported data.[4]

(b) (S)-Isomer. In the same manner as described above, (S)-16 (327 mg, 1.13 mmol) gave 287 mg (88%) of (S)-1, whose spectral data were identical with those of (R)-1, m.p. 89-91 °C; $[\alpha]_D^{24}$ -131° (c=0.25, MeOH). (Found: C, 70.65; H, 7.61; N, 4.82. Calc for $C_{17}H_{21}O_3N$: C, 71.06; H, 7.37; N, 4.87%).

Trichostatin A 2

(a) O-(2-Methoxypropyl)hydroxylamine 20. A suspension of N-hydroxyphthalimide 18 (15 g, 93 mmol) and 2-methoxypropene (20 ml, 209 mmol) in acetonitrile (300 ml) was stirred at room temp for 7 days without any catalyst. The mixture was diluted with sat $NaHCO_3$ soln and concentrated in vacuo. The residue was extracted with EtOAc. The EtOAc soln was washed with water, brine, dried (K_2CO_3) and concentrated in vacuo. Recrystallization from ether gave 15 g (69%) of O-(2-methoxypropyl)-N-hydroxyphthalimide 19 as yellow prisms, m.p. 102-104 °C. This protected phthalimide 19 (15 g, 64 mmol) was treated with hydrazine (6.4 ml, 132 mmol) in MeOH (32 ml) and CH_2Cl_2 (80 ml). After stirring at room temp for 2 h, the reaction mixture was filtered. The filtrate was concentrated in vacuo and to it was added 10% NaOH soln (130 ml). The mixture was then extracted with ether. The ether soln was washed with water, brine, dried ($MgSO_4$) and concentrated in vacuo. The residue was distilled using a Vigreaux column (5 cm) to give 4.37 g (45%) of O-(methoxypropyl)hydroxylamine 20 as a colorless oil, b.p. 70-73 °C/103 Torr, n_D^{21} 1.4118; νmax 3330 (s), 3250 (m), 3000 (s), 2950 (s), 2850 (m), 1600 (m), 1460 (m), 1380 (s), 1220 (s), 1190 (s), 1150 (s), 1070 (s), 830 (s) cm^{-1}; δ ($CDCl_3$) 1.31 (6H, s), 3.20 (3H, s), 4.90 (2H, br s), (Found: C, 46.00; H, 10.51; N, 12.95. Calc for $C_4H_{11}O_2N$: C, 45.70; H, 10.55; N, 13.32%).

(b) (R)-Isomer. To an ice-cooled soln of (R)-trichostatic acid (1, 332 mg, 1.16 mmol) and Et_3N (233 mg, 2.31 mmol) in dry THF (5ml) was added $ClCO_2Et$ (120 mg, 1.27 mmol) in dry THF (5 ml). After 10 min, it was added O-(2-methoxypropyl)-hydroxylamine (0.24 ml, 2.31 mmol) in THF (5 ml). The soln was stirred for 5 min at 0 °C. It was then poured into ice-water and was extracted with EtOAc. The organic layer was washed with water, brine, dried ($MgSO_4$) and concentrated in vacuo to give crude O-(2-methoxypropyl)-trichostatin A (350 mg). The crude sample was purified quickly using medium pressure SiO_2 column chromatography (Yamamo Kagaku Co. Ltd., ID-22, EtOAc) to give 141 mg (33%) of the product. It was subjected to the next reaction immediately. To a soln of the protected hydroxamate (92 mg, 0.25 mmol) in MeOH (5 ml) was added Amberlyst 15 (25 mg) and the mixture was stirred at 45 °C for 1 h. It was filtered and the filtrate was concentrated in vacuo to give 60 mg (81%) of (R)-trichostatin A (2). Recrystallization from EtOAc-MeOH gave a pure sample as yellow fine needles, m.p. 146-150 °C(dec.) [lit. 150-151 °C[2], 172-174 °C[13]]; $[\alpha]_D^{22}$ +96° (c=0.31, MeOH) [lit. +63° (c=0.1, MeOH)[13], +77° (c=0.23, EtOH) [lit. +62.8° (c=1.007 ,EtOH)[1]]; νmax ($CHCl_3$ soln) 3250 (m, br), 1660 (s), 1600 (s), 1370 (s), 1190 (s), 1170 (s), 980 (m), 820 (m) cm^{-1}; δ ($CDCl_3$: CD_3OD 5:1) 1.30 (3H, d, J=6.8 Hz), 1.91 (3H, d, J=0.9 Hz), 3.08 (6H, s), 4.40 (1H, dq, J=9.2 and 6.8 Hz), 5.79 (1H, d, J=15.5 Hz), 5.97 (1H, d, J=9.2 Hz), 6.65 (2H, d, J=9.0 Hz), 7.20 (1H, d, J=15.5 Hz), 7.64(2H, d, J=9.0 Hz); ^{13}C NMR δ (125 MHz, Bruker AM-500, $CDCl_3$:CD_3OD=5:1) 12.6, 17.9, 40.0, 40.9, 111.0, 115.7, 123.8, 130.9, 133.0, 140.3, 145.3, 154.0, 165.5, 199.8 ; UV max(ε)(nm), (c=1.7x10^{-5}, MeOH) 342 (27000), 264 (24100), 250 (sh, 23000); CD [Θ](nm)(c=0.0231 g/l, 7.64x10^{-5} M/l, MeOH) 340 (350 nm), 0 (328), -196 (310), 0 (270), 98 (260), 0 (252), -262 (240), 0 (210). Found: C, 67.37; H, 7.43; N, 9.14. Calc for $C_{17}H_{22}O_3N_2$: C, 67.52; H, 7.33; N, 9.27%). The IR[1], ^1H-, ^{13}C-NMR[13] and UV[1,13] spectra were identical with the reported data.

(c) (S)-Isomer. In the same manner as described above, (S)-1 (191 mg, 0.67 mmol) gave 79 mg (39%) of (S)-2 whose spectral

data were identical with those of (R)-2, m.p. 143-144 °C; $[\alpha]_D^{17}$ -82° (c=0.34, MeOH). (Found: C, 67.17; H, 7.38; N, 9.16. Calc for $C_{17}H_{22}O_3N_2$: C, 67.52; H, 7.33; N, 9.27%).

Determination of the enantiomeric purities of methyl 2-methyl-3-hydroxypropanoate (R)-4
The (R)-MTPA ester of both the enantiomers of (R)-4 were subjected to an HPLC analysis (Senshu Pack SiO_2 1251-N, n-hexane/THF:MeOH=8000:1000:1, 1 ml/min, 4.6 mm x 250 mm, detected at 254 nm). Rt was 46.5 min for (R)-alcohol and 58.0 min for (S)-alcohol. The enantiomeric purity was 99% for both the enantiomers of 4.

Determination of the enantiomeric purities of trichostatic acid 1.
To a sola of trichostatic acid 1 in MeOH was added excess CH_2N_2 in ether and the mixture was left to stand for 1 h at room temp. The mixture was subjected to an HPLC analysis. (CHIRALCEL-OB, Daicel Chemical Industries Ltd., 90% MeOH, 0.6 ml/min; detected at 254 nm) Rt was 21.0 min for (S)-1, 33.0 min for (R)-1. The enantiomeric purities were calculated from peak area of the both enantiomers. Both the enantiomers were estimated to be over 98% e.e.

Determination of the enantiomeric purities of trichostatin A 2
To a sola of trichostatin A (2) in MeOH was added excess CH_2N_2 in ether and the mixture was left to stand for 1 h at room temp. The mixture was subjected to an HPLC analysis. (CHIRALCEL-OB, n-hexane:2-propanol, 9:1, 0.6 ml/min, detected at 254 nm) Rt was 30 min for (R)-2 and 37 min for (S)-2. The (S)-form in (R)-2 could not be separated cleary because of the large tailing peak of (R)-2. (R)-2 in (S)-2 was separated. The enantiomeric purity of (S)-trichostatin A 2 was at least 93% e.e.

Acknowledgement— We thank Dr. H. Tsuji and Dr. M. Kobayashi of Shionogi and Co., Ltd (Osaka) for kindly sending to us the natural trichostatic acid (1) and trichostatin A (2). We are grateful to Prof. T. Beppu and Dr. M. Yoshida for helpful discussion. Our thanks are due to Daicel Chemical Industries, Ltd. for the chiral packing HPLC columns. Financial support by Japan Tobacco Inc. is acknowledged with thanks.

REFERENCES

1 H. Tsuji, M. Kobayashi, K. Nagashima, Y. Wakisaka and Koizumi, J. Antibiot., 29, 1 (1976).
2 H. Tsuji, M. Kobayashi, J. Antibiot., 31, 939 (1978).
3 M. Yoshida, Y. Iwamoto, T. Ozumi and T. Beppu, Agric. Biol. Chem., 49, 563 (1985).
4 H. Moricka, M.Ishihara, M. Takamura, K. Hirayama, R. Suzuki, Y. Komoda and H. Shibai, Agric. Biol. Chem., 49, 1365 (1985).
5 M. Yoshida, S. Nomura and T. Beppu, Cancer Res., 47, 3688 (1987).
6 I. Fleming and J. Iqbal, Tetrahedron, 38, 841 (1983).
7 H. Moricka, M. Ishihara, M. Takamura, H. Shibai and Y. Komoda, Agric. Biol. Chem., 52, 583 (1988).
8 J. A. Dale and H. S. Mosher, J. Am. Chem. Soc., 95, 512 (1973).
9 A. J. Mancuso, S. L. Huang and D. Swern, J. Org. Chem., 43, 2480 (1978).
10 A. Mandel, J. Organometal Chem., 6, 97 (1966).
11 J. R. Parikh and W. E. Doering, J. Am. Chem. Soc., 89, 5505 (1967).
12 A. B. Smith, III and T. L. Leenay, Tetrahedron lett., 29, 49 (1988).
13 H. Moricka, M. Ishihara, M. Takamura, H. Shibai and Y. Komoda, Agric. Biol. Chem., 52, 251 (1988).
14 K. Otoguro, H. Oiwa, Y. Iwai, H. Tanaka and S. Ōmura, J. Antibiot., 41, 461 (1988).