Synthesis of *N*-Boc-Statine and *epi*-Statine

Peter G.M. Wuts,* Sterling R. Putt

Chemical Process and Development, 1500-230-4, The Upjohn Co, Kalamazoo, MI 49001, USA

The amino acid statine and its C-3 epimer have been prepared stereoselectively as their *N*-*tert*-butoxycarbonyl (*N*-Boc) derivatives **8** and **9** from (*S*)-Boc-leucinal **(1)**, and (*S*)-**(2)** and (*R*)-1,1,2-triphenyl-2-acetoxyethanol **(3)**, respectively.

Statine [(3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid], originally isolated from Pepstatin, has proven to be an important amino acid because its incorporation into a variety of peptide derivatives has yielded a number of very active renin inhibitors.[1-3] Although several syntheses of statine have been reported in the literature,[2,4,5] they generally lack stereoselectivity and/or efficiency. In this paper we describe an efficient, stereoselective and simple synthesis of both *N*-Boc statine **(8)** and its C-3 epimer **9** which is based on Braun's observation that the magnesium enolates of (*S*)-**(2)** or (*R*)-2-acetoxy-1,1,2-triphenylethanol **(3)** are highly effective in introducing the acetate unit with excellent enantioselectivity.[6] Thus the magnesium enolate of (*S*)-**2** was prepared and treated with (*S*)-Boc-leucinal **(1)** to afford a diastereoisomeric mixture of esters **4** and **5**. When the reaction was run at −70 °C a 8.5:1 ratio of isomers was obtained, which could be improved to 11.3:1 running the reaction at Braun's recommended temperature of −110 °C. Alkaline hydrolysis of the mixture of esters **4** and **5** affords (3*S*,4*S*)-Boc-statine **(8)** as a 11.3:1 mixture of diastereoisomers in an overall yield of 48% after crystallization (Table). Alternatively, when the reaction is run at −70 °C using the lithium enolate of **2** an initial ratio of 8:1 is obtained for the diastereoisomeric esters **4** and **5**. Hydrolysis of the mixture and crystallization affords an 81% yield of Boc-statine **(8)** and its epimer **9** in a 9.2:1 ratio. With the lithium enolate there is little advantage to run the reaction at −110 °C, since there is no significant improvement in the ratio or the yield. During this work we found that it was relatively difficult to remove the (3*R*,4*S*)-isomer **9** by crystallization since this isomer is much more crystalline than Boc-statine **(8)**. However, if the mixture is converted to a dicyclohexylamine salt it is easily upgraded by crystallization from 2-propanol affording ratios > 60:1.

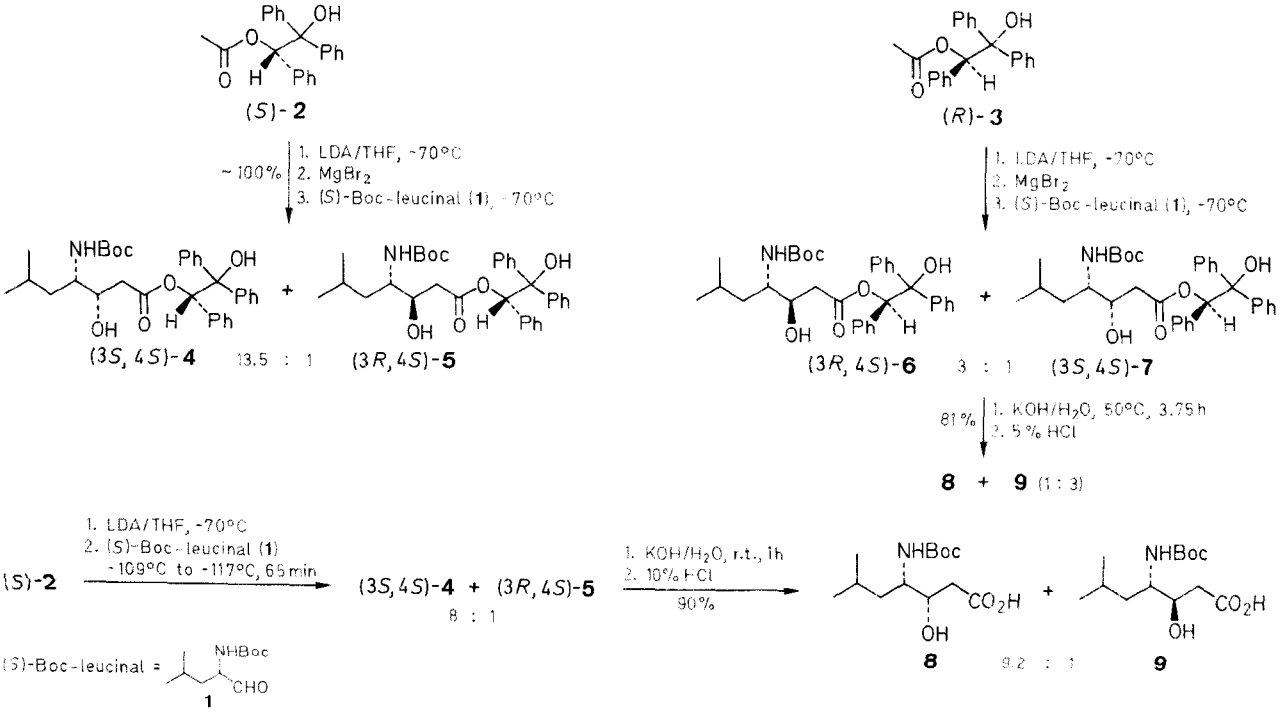
In an analogous fashion, coupling the enantiomeric magnesium enolate of (*R*)-**3** with (*S*)-Boc-leucinal **(1)** affords a diastereoisomeric mixture of esters **6** and **7** in a 3:1 ratio. From this result it is clear that we are dealing with the mismatched pair of aldehyde and enolate. Alkaline hydrolysis and crystallization gives *epi*-Boc-statine **(9)** in 43% yield as a 20:1 mixture of diastereoisomers. It is interesting to note that in this case the very poor ratio of diastereoisomers is easily upgraded to give the highly crystalline (3*R*,4*S*)-isomer **9**, which explains why removal of this isomer from the (3*S*,4*S*)-isomer **8** proved to be so troublesome.

In addition to developing one of the most efficient synthesis of statine,[7] these results show that the enolate of 2-acetoxy-1,1,2-triphenylethanol (**2** and **3**) not only gives excellent enatioselectivity but also provides excellent diastereoselectivity in its reaction with chiral aldehydes. Moreover, this is one of the best reagents to date for the direct diastereoselective addition of an acetate unit to an α-chiral aldehyde. The advantages of this approach

Table. Reaction Conditions for the Synthesis of Boc-Statine

Enolate	Temperature (°C)	Ester (4/5) Ratio	Boc-Statine (8/9) Ratio	Yield (%)
Magnesium	−70	8:1	20:1	48[a]
Magnesium	−110	11:1	10:1	47[b]
Lithium	−70	8:1	10:1	81[c]
Lithium	−110	–	11:1	76[c]

[a] Crystallized from Et_2O/hexane.
[b] Crystallized from *tert*-butyl methyl ether/hexane.
[c] Crystallized from Et_2O/cyclohexane.



are that both Boc-statine **(8)** and *epi*-Boc-statine **(9)** may readily be prepared with excellent efficiency and without chromatography, which is particularly important in an industrial environment.

THF was dried by distilling from sodium benzophenone ketyl. (*S*)-Boc-leucinal **(1)** was prepared according to literature procedure.[8] ^{1}H-NMR spectra were recorded on a Bruker 300 MHz instrument.

(3*S*,4*S*)-Boc-Statine 1,1,2-Triphenylethyl Ester (4):
A 0.62 M THF solution of LDA (60 mL, 37.4 mmol, prepared from diisopropylamine and butyllithium) is added to a stirred suspension of (5)-**2** (5.0 g, 15.06 mmol) in THF (65 mL) at −78 °C via cannula over 12 min maintaining the temperature at −70 °C. When the addition is complete, the suspension is warmed to 0 °C and stirred for 30 min to give a light orange slightly hazy solution. In a 500 mL 3-neck round bottomed flask fitted with a low temperature thermometer, three way stopcock for admission of dry N_2, and pressure equalizing dropping funnel, a mixture of Mg turnings (0.73 g, 30.1 mmol) in THF (15 mL) and 1,2-dibromoethane (2.8 mL, 32 mmol) is warmed until the Grignard reaction starts. It is stirred gently and heated as necessary until the reaction is complete. The heavy white solid is diluted with THF (100 mL) and cooled to −78 °C. The above orange enolate solution is added dropwise to the $MgBr_2$ suspension at −78 °C over 8 min (temperature is maintained at −72 °C to −74 °C) and the mixture is stirred at −74 °C for 1 h. A solution of (*S*)-Boc-Reucinal (**1**; 2.94 g, 13.64 mmol) in THF (50 mL) is added dropwise over 9 min (temperature is maintained between −72 °C and −74 °C). The mixture is stirred at −75 °C for 42 min, and quenched with sat. NH_4Cl solution (30 mL), and warmed to r.t. The suspension is poured into water (150 mL) and extracted with CH_2Cl_2 (2 × 100 mL), dried ($MgSO_4$), and concentrated to a yellow glass (8.35 g, 111.8 %). HPLC analysis shows a 8.5 to 1 ratio of the (3*S*,4*S*)- and (3*R*,4*S*)-diastereoisomers. The crude product is purified by flash chromatography on EM Silica Gel G60 (560 g, 30 % EtOAc/cyclohexane) to give 7.48 g (100 %) of a white solid with a diastereoisomer ratio of 13.5 to 1. This material is recrystallized from hot toluene (40 mL) to give white crystals (3.1 g) with a 17:1 ratio of diastereoisomers. An analytical sample is obtained by chromatography and by two subsequent recrystallization from 10 % EtOAc/heptane to give only one diastereoisomer (3*S*,4*S*) as detected by HPLC; yield: 0.51 g (22.5 %); mp 187–188 °C; $[\alpha]_D = +138.5°$ ($c = 0.31$, MeOH).

$C_{33}H_{41}NO_6$	calc.	C 72.37	H 7.55	N 2.56
(547.7)	found	72.47	7.74	2.70

IR (KBr): ν = 3418, 3415, 3410, 2955, 1733, 1698, 1510, 1497, 1450, 1395, 1367, 1339, 1281, 1261, 1250, 1165, 1065, 1045, 1039, 1033, 751, 735, 699 cm^{-1}.

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.84 (d, 3 H, J = 6.82 Hz); 0.86 (d, 3 H, J = 6.64 Hz); 1.13 (m, 1 H); 1.43 (s, 9 H); 1.48 (m, 1 H); 2.42 (d, 2 H, J = 6.44 Hz); 2.84 (d, 1 H, J = 3.56 Hz); 3.43 (br s, 2 H); 3.73 (m, 1 H); 4.64 (d, 1 H, J = 9.84 Hz); 6.71 (s, 1 H); 7.32 (m, 15 H).

^{13}C-NMR ($CDCl_3$/TMS): δ = 22.31, 23.12, 24.79, 28.38, 39.49, 41.51, 51.87, 69.90, 79.07, 79.34, 80.21, 126.27, 126.36, 127.02, 127.39, 127.47, 127.77, 127.98, 128.34, 128.52, 135.61, 142.60, 144.79, 156.11, 171.47.

HRMS: m/z calc. for $C_{33}H_{41}NO_6$: 548.3012; found: 548.3009 (M^+).

(3*S*,4*S*)-*N*-Boc-4-amino-3-hydroxy-6-methylheptanoic Acid [3*S*,4*S*)-Boc-Statine, 8]:
A solution of LDA prepared by the addition of BuLi (1.6 M in hexane; 15.6 mL, 25 mmol) to diisopropylamine (4.2 mL, 30 mmol) in THF (20 mL) is added to a stirred suspension of (*S*)-**2** (3.326 g, 10 mmol) in THF (50 mL) at −78 °C over 5 min. The mixture is warmed to 0 °C and held there until an orange solution is obtained (5 min) and cooled to −72 °C. Anhydrous Et_2O (95 mL) is added and the solution is cooled to −110 °C and (*S*)-Boc-leucinal (**1**; 2.47 g, 11.5 mmol) in Et_2O (8 mL) is added dropwise over 5 min (temperature is maintained between −109 °C and −110 °C). The mixture is stirred at −109 °C to −117 °C for 65 min at which time it is quenched with the dropwise addition of sat. NH_4Cl solution (20 mL), and warmed to r.t. The suspension is poured into water (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL), dried ($MgSO_4$), and concentrated to an orange foam (6.65 g, 121.4 %). A solution of the Boc-statine esters **4/5** (8:1) thus obtained is dissolved in MeOH (100 mL) and treated with KOH (2.82 g, 50.19 mmol) in water (50 mL) stirred at r.t. for 1 h. The suspension is poured into water (100 mL) and extracted 3 with CH_2Cl_2 (3 × 100 mL), dried ($MgSO_4$), and concentrated to a gold solid residue of recovered (*S*)-1,1,2-triphenyl ethylene glycol. The aqueous layer (pH = 13.3) is acidified with 10 % HCl to pH 2 and extracted with methyl *tert*-butyl ether (3 × 100 mL), dried ($MgSO_4$), and concentrated to a white solid residue (2.48 g, 90 %). HPLC analysis shows a 9.2 to 1 ratio of diastereoisomers. The crude Boc-statine is dissolved in hot Et_2O (30 mL) and then diluted with cyclohexane (30 mL). The solvent is removed by distillation and upon cooling crystallization begins. After keeping overnight at r.t., the white crystalline mass is filtered and washed with 10 % methyl *tert*-butyl ether in hexane (3 × 10 mL) and dried to constant weight of 2.10 g (76 %). HPLC analysis shows a ratio of diastereoisomers of 11.3 to 1; mp 115–116 °C; Lit.[5] mp 117–118 °C; $[\alpha]_D = -36.9°$ (c = 0.31, MeOH); Lit.[5] $[\alpha]_D^{24} = -39.6°$ (c = 0.31, MeOH).

IR (KBr): ν = 3380, 2955, 2933, 1700, 1682, 1520, 1365, 1274, 1233, 1177 cm^{-1}.

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.92 (s, 3 H); 0.94 (s, 3 H); 1.31 (m, 2 H); 1.45 (s, 9 H); 1.60 (m, 1 H); 2.55 (m, 2 H); 3.64 (m, 1 H); 4.02 (m, 1 H); 4.84 (d, 1 H, J = 9.69); 5.88 (d, 1 H, J = 9.72).

^{13}C-NMR (DMSO-d_6/TMS): δ = 21.96, 23.37, 24.56, 28.34, 38.79, 39.00, 52.12, 69.35, 77.60, 155.67, 173.31.

HRMS: M^+ calc. for $C_{13}H_{25}O_5$: 276.1811; found: 276.1794.

(3*R*,4*S*)-*N*-Boc-4-amino-3-hydroxy-6-methylheptanoic Acid [(3*R*,4*S*)-Boc-*epi*-Statine, 9]:

(3R,4S)-Boc-Statine 1,1,2-Triphenylethyl Ester **(6)**: A solution of LDA prepared by the addition of BuLi (1.6 M in hexane, 15.6 mL, 25 mmol) to diisopropylamine (4.2 mL, 30 mmol) in THF (20 mL) is added dropwise to a stirred suspension of (*R*)-**3** (3.33 g, 10.02 mmol) in THF (50 mL) at −78 °C over 5 min. The solution is warmed to 0 °C and held there until an orange solution is obtained (5 min) and then cooled to −72 °C. In a 500 mL 3 necked round bottomed flask fitted with a low temperature thermometer, three way stopcock for admission of dry nitrogen, and pressure equalizing dropping funnel, a mixture of Mg turnings (0.49 g, 20.2 mmol) in THF (20 mL) and 1,2-dibromoethane (2.6 mL, 30 mmol) is warmed until the Grignard reaction starts. The mixture is stirred gently and heated as necessary until the reaction is complete. The heavy white solid suspension is diluted with Et_2O (120 mL) and cooled to −72 °C. The orange enolate solution of (*R*)-**3** is added to the $MgBr_2$ suspension and then stirred for 1.25 h. (*S*)-Boc-leucinal (**1**; 2.4 g, 11.1 mmol) in Et_2O (6 mL) is added dropwise over 5 min at −70 °C. The mixture is stirred at −70 °C for 40 min at which time it is quenched by the dropwise addition of sat. NH_4Cl solution (20 mL) and warmed to r.t. The suspension is poured into water (100 mL) and extracted with CH_2Cl_2 (1 × 200 mL, and 2 × 100 mL), dried ($MgSO_4$), and concentrated to yellow solid residue; yield: 7.86 g (43 %). Normally this crude ester can be saponified to give a mixture of Boc-*epi*-statine **(9)** and Boc-statine **(8)**. To obtain a pure sample of (3*R*,4*S*) ester **6**, the crude product is rigorously purified by flash chromatography; mp 190–191 °C; $[\alpha]_D = +102.7°$ (c = 0.31, MeOH).

$C_{33}H_{41}NO_6$	calc.	C 72.37	H 7.55	N 2.56
(547.7)	found	72.06	7.71	2.63

IR (KBr): ν = 3287, 2958, 1719, 1695, 1693, 1672, 1548, 1496, 1450, 1394, 1368, 1336, 1323, 1297, 1278, 1247, 1168, 1144, 1046, 1035, 1019, 1001, 754, 735, 698, 644, 605 cm^{-1}.

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.90 (dd, 6 H, J = 6.56, 4.78 Hz); 1.25 (m, 2 H); 1.45 (s, 9 H); 1.63 (m, 1 H); 2.42 (d, 2 H, J = 6.22); 2.87 (d, 1 H, J = 4.12 Hz); 3.58 (s, 1 H); 3.66 (m, 1 H); 3.82 (m, 1 H); 4.60 (d, 1 H, J = 8.97 Hz); 6.72 (s, 1 H); 7.15 (m, 10 H); 7.29 (t, 1 H, J = 7.29 Hz); 7.37 (t, 2 H, J = 7.46 Hz); 7.61 (d, 2 H, J = 7.57 Hz).

^{13}C-NMR ($CDCl_3$/TMS): δ = 21.50, 23.65, 24.63, 28.36, 38.08, 38.64, 52.45, 71.01, 79.32, 79.60, 80.14, 126.24, 126.31, 126.96, 127.32, 127.44, 127.72, 128.31, 128.55, 135.59, 142.60, 144.79, 155.96, 171.00.

HRMS: M^+ calc. for $C_{33}H_{41}NO_6$: 548.3012; found: 548.3036 (M^+).

Hydrolysis of (3R,4S)-6 to (3R,4S)-Boc-epi-statine **(9)**: A solution of the ester mixture (3*R*,4*S*)-**6** and (3*S*,4*S*)-**7** in MeOH (100 mL) is stirred with a solution of KOH (5.74 g, 102.2 mmol) in water (50 mL) at 50 °C for 3.75 h. The suspension is cooled to r.t. and MeOH is removed on a rotary evaporator. The resulting suspension is poured into water (80 mL) and extracted with CH_2Cl_2 (3 × 100 mL), dried ($MgSO_4$), and the solvent removed on a rotary evaporator to afford a gold solid residue of recovered (*R*)-1,1,2-triphenyl ethylene glycol. The aqueous layer (pH = 13.9) is acidified with 5 % HCl to pH 2 and extracted with methyl *tert*-butyl ether (3 × 100 mL) dried ($MgSO_4$), and the solvent removed on a rotary evaporator to a yellow oil, which crystallizes upon standing (2.23 g, 81 %). HPLC analysis shows a 1:3 ratio of diastereoisomers. The crude Boc-*epi*-statine **(9)** is recrystallized from a

mixture of hot Et_2O (25 mL) and cyclohexane (40 mL). After standing for 2 d at r.t., the white crystalline mass is filtered and washed with Et_2O/hexane (1:1, 2 × 10 mL) and dried to constant weight; yield: 1.18 g (43%). HPLC analysis shows a ratio of diastereoisomers of 1 to 19.4; mp 132–133°C; Lit.[5] mp 135–136°C; $[\alpha]_D - 25.6°$ ($c = 0.31$, MeOH); Lit.[5] $[\alpha]_D^{24} - 27.6°$ ($c = 0.31$, MeOH).

IR (KBr): ν = 3356, 3284, 2985, 2952, 2936, 1714, 1690, 1531, 1275, 1179, 1074, 1000, 650 cm^{-1}.

^{1}H-NMR ($CDCl_3$/TMS): δ = 0.93 (dd, 6H, J = 6.92, 8.17 Hz); 1.32 (dd, 2H, J = 6.56, 7.70); 1.45 (s, 9H); 1.67 (m, 1H); 2.50 (d, 2H, J = 5.99 Hz); 3.72 (m, 1H); 4.02 (m, 1H); 4.66 (d, 1H, J = 8.10 Hz); 5.63 (m, 1H).

^{13}C-NMR ($CDCl_3$/TMS): δ = 21.58, 23.48, 24.79, 28.31, 37.34, 39.01, 53.05, 71.49, 80.34, 156.93, 175.89.

HRMS: M^+ calc. for $C_{13}H_{25}NO_5$: 276.1811; found: 276.1794 (M^+).

Received: 20 March 1989; revised: 14 July 1989

(1) Boger, J., Lohr, N.S., Ulm, E.H., Poe, M., Blaine, E.H., Fanelli, G.M., Lin, T.-Y., Payne, L.S., Schorn, T.W., La Mont, B.I., Vassil, T.C., Stabilito, I.I., Veber, D.F., Rich, D.H., Bopari, A.S. *Nature* **1983**, *303*, 81.

(2) Rittle, K.E., Homnick, C.F., Ponticello, G.S., Evans, B.E. *J. Org. Chem.* **1982**, *47*, 3016.

(3) Boger, J. *Proc. of the 8th Am. Peptide Symp.*, p. 569, Tucson, 1983.

(4) For previous syntheses of statine and there reported overall yields:
Morishima, H., Takita, T., Umezawa, H. *J. Antibiot.* **1973**, *26*, 115 (No yield).
Steulmann, R. and Klostermeyer, H. *Liebigs Ann. Chem.* **1975**, 2245 (18–24%).
Katsuki, T., Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 3287 (33.9%, a mixture of diastereoisomers at C-3).
Kirihata, M., Tokumori, H., Ichimoto, I., Ueda, H. *Nippon Nogei Kagaku Kaishi* **1978**, *52*, 135; *C.A.* **1978**, *89*, 146371 (No yield).
Kirihata, M. *Bull. Univ. Osaka Prefect. Ser. B* **1981**, *33*, 135; *C.A.* **1982**, *96*, 143271 (No yield).
Liu, W.S., Glover, G.I. *J. Org. Chem.* **1978**, *43*, 754. (34%, mixture of diastereoisomers at C-4).
Danishefsky, S., Kobayashi, S., Kerwin, Jr., J.F. *J. Org. Chem.* **1982**, *47*, 1981 (No yield).
Rittle, K.E., Homnick, C.F., Ponticello, G.S., Evans, B.E. *J. Org. Chem.* **1982**, *47*, 3016 (17.7%).
Hayon, A.F., Fehrentz, J.A., Chapleur, Y., Castro, B. *Bull. Soc. Chim. Fr. Part II*, **1983**, *207* (47% yield of a 70/30 mixture of epi-boc-statine methyl ester).
Rague, B., Fehrentz, J.A., Guegan, R., Chapleur, T., Castro, B. *Bull. Soc. Chim. Fr.* **1983**, 230 (49.5% yield of a 70:30 mixture of *epi*-Boc-statine methyl ester).
Castro, B., Guegan, R. *Fr. Demande FR*, 2531076 (1984); *C.A.* **1984**, *101*, 23943 (No yield).
Woo, P.W.K. *Tetrahedron Lett.* **1985**, *26*, 2973 (24%).
Dufour, M.N., Jouin, P., Poncet, J., Pantaloni, A., Castro, B. *J. Chem. Soc. Perkin Trans. 1* **1986**, 1895 (24%).
Kogen, H., Nishi, T. *J. Chem. Soc. Chem. Commun.* **1987**, *311* (55.8%).
Jouin, P., Castro, B. *J. Chem. Soc. Perkin Trans. 1* **1987**, 1177 (55.4%).
Kano, S., Yokomatsu, T., Iwasawa, H., Shibuya, S. *Chem. Lett.*, **1987**, 1531 (23.7%).
Andrew, R.G., Conrow, R.E., Elliott, J.D., Johnson, W.W., Ramezani, S. *Tetrahedron Lett.* **1987**, *28*, 6335 (21.4%).
Maibaum, J., Rich, D.H. *J. Org. Chem.* **1988**, *53*, 869 (27%).
Kinoshita, M., Aburaki, S., Hagiwara, A., Imai, J. *J. Antibiot.* **1973**, *26*, 249 (No yield).
Kinoshita, M., Hagiwara, A., Aburaki, S. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 570 (8.5%).
Shi, H., Shi, Q., Hou, J., Yang, F., Li, D. *Yiyao Gongye* **1984**, 8. *C.A.* **1984**, *101* 231019 (No yield).
Sakaitani, M., Ohfune, Y. *Tetrahedron Lett.* **1987**, *28*, 3987 (38.3%).
Sakaitani, M., Hori, K., Ohfune, Y. *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu* **1986**, *28*, 526. *C.A.* **1987**, *107*, 78206 (Review).
Ofuna, Y., Sakaitani, M. *C.A.* **1988**, *108*, 167967 (No yield).
Yabe, Y., Furumoto, H., Nishi, T. *Japan Kokai* JP 62205070 (1987), Sankyo Co. Ltd.; C.A. **1988**, *108*, 167965 (No yield).
Mulzer, J., Buettelmann, B., Muench, W. *Liebigs Ann. Chem.* **1988**, 445 (13%).
Kunieda, T., Ishizuka, T., Higuchi, T., Hirobe, M. *J. Org. Chem.* **1988**, *53*, 3381 (20.1%).
Kano, S., Yuasa, Y., Yokomatsu, T., Shibuya, S. *J. Org. Chem.* **1988**, *53*, 3865 (36.1%).

(5) Rich, D.H., Sun, E.T., Boparai, A.S. *J. Org. Chem.* **1978**, *43*, 3624 (40%).

(6) Braun, M., Devant, R. *Tetrahedron Lett.* **1984**, *25*, 5031.

(7) A report relating similar work was published while our work was in progress, but these authors report only on a variety of statine analogs and not on statine itself: Devant, R.M., Radunz, H.-E. *Tetrahedron Lett.* **1988**, *29*, 2307.

(8) (*S*)-Boc-leucinal was freshly prepared prior to use because of its tendency to racemize. For preparation see: Hamada, Y., Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 1921.
Hamada, Y., Shioiri, T. *Tetrahedron Lett.* **1982**, *23*, 1193.