Lavendomycin: Total Synthesis and Assignment of Configuration[1]

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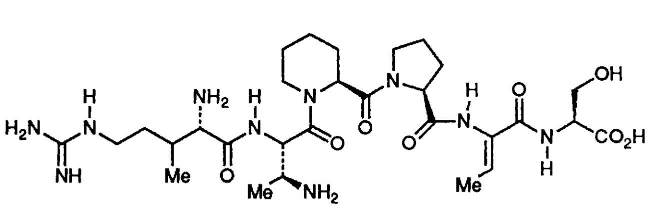
(−)-Lavendomycin, a highly potent antibiotic hexapeptide with very low toxicity, isolated from culture filtrates of *Streptomyces lavendulae* subsp. *brasilicus* has been synthesized.

Lavendomycin (**1a**)[2] has been isolated from culture filtrates of *Streptomyces lavendulae* subsp. *brasilicus*. It is a hexapeptide exhibiting a very low toxicity ($LD_{50} > 2$ g kg^{-1} on intraperitoneal injection in mice) and a high antibiotic activity towards Gram-positive bacteria both *in vivo* and *in vitro*. The configurations of the amino acids and their sequence in the hexapeptide have been determined by total hydrolysis and Edman degradation.

Up to the last step in the synthesis one guanidino, two amino, and the carboxy groups have to be masked and were protected in the form of the respective t-butoxycarbonyl (Boc), adamantyloxycarbonyl (Adoc),[3] and allyl (All) derivatives.[4] The hydroxy group of serine remained unprotected throughout all of the steps. The use of masks cleavable by hydrogenolysis was excluded by the presence of double bonds in the didehydroamino acid and allyl ester moieties. The fluorenylmethoxycarbonyl (Fmoc)[5] and trichloroethyl (Tce)[6] masking groups were found to be compatible with all protecting groups and functions involved in the construction of the peptide.



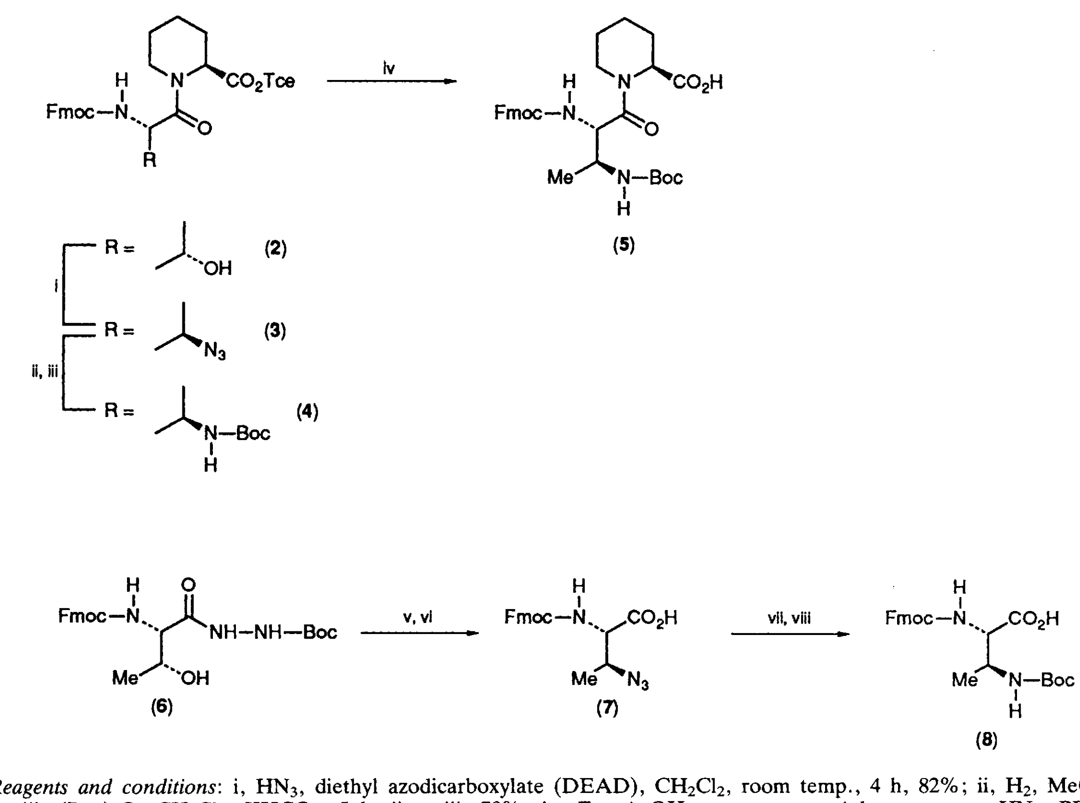
(**1a**)

In addition to (*S*)-proline and (*S*)-serine, the peptide also contains the non-protein amino acids (*S*)-pipecolinic acid, didehydroaminobutyric acid, (*S*,*S*)-α,β-diaminobutyric acid, and (2*S*)-3-methylarginine; the configuration of the last mentioned acid at the 3-position has not been elucidated.

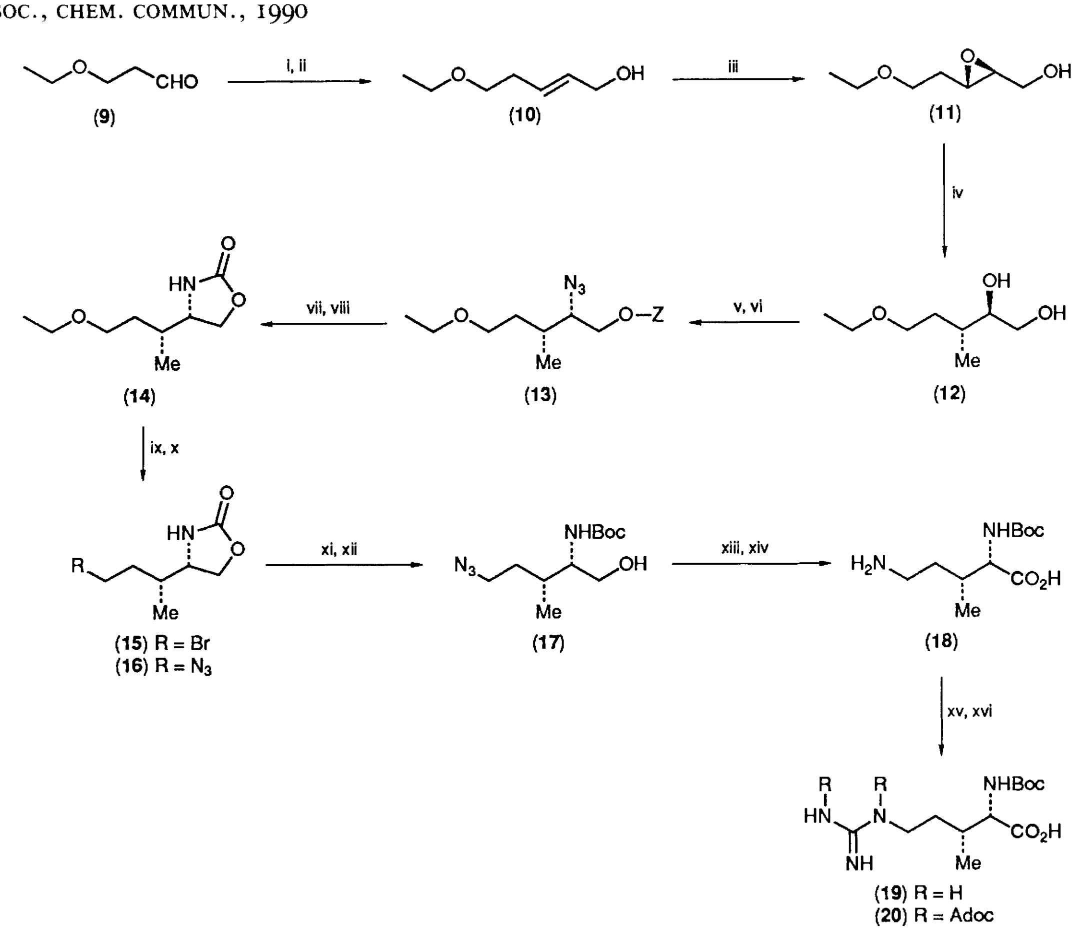
In the course of the total synthesis of lavendomycin, we have now elaborated syntheses of the latter three amino acids as described below.

The formation of (*Z*)-didehydroaminobutyric acid (DDAB) was combined with the construction of the tripeptide Boc-Pro-DDAB-Ser-OAll utilising 2 mol equiv. of disuccinimidyl carbonate (DSC)[7] for simultaneous elimination of water and peptide bond formation.

(*S*,*S*)-2,3-Diaminobutyric acid, previously only accessible with difficulty,[8] was prepared with the help of the Mitsunobu reaction[9] from the threonine derivatives Fmoc-Thr-Pip-OTce (**2**) and Fmoc-Thr-NH-NH-Boc (**6**) with free hydrazoic acid (Scheme 1). The reaction must be carried out with threonine



Scheme 1. *Reagents and conditions*: i, HN_3, diethyl azodicarboxylate (DEAD), CH_2Cl_2, room temp., 4 h, 82%; ii, H_2, MeOH, Lindlar catalyst, 6 h; iii, $(Boc)_2O$, CH_2Cl_2, $KHCO_3$, 5 h, ii + iii: 70%; iv, Zn, AcOH, room temp., 4 h, quant.; v, HN_3, PPh_3, DEAD, CH_2Cl_2, room temp., 6 h, 75%; vi, 6 M HCl, 90 °C, 8 h, 76%; vii, H_2, Pd, MeOH, 5 h; viii, H_2O, dioxane, $KHCO_3$, $(Boc)_2O$, 4 h, vii + viii: 70%.



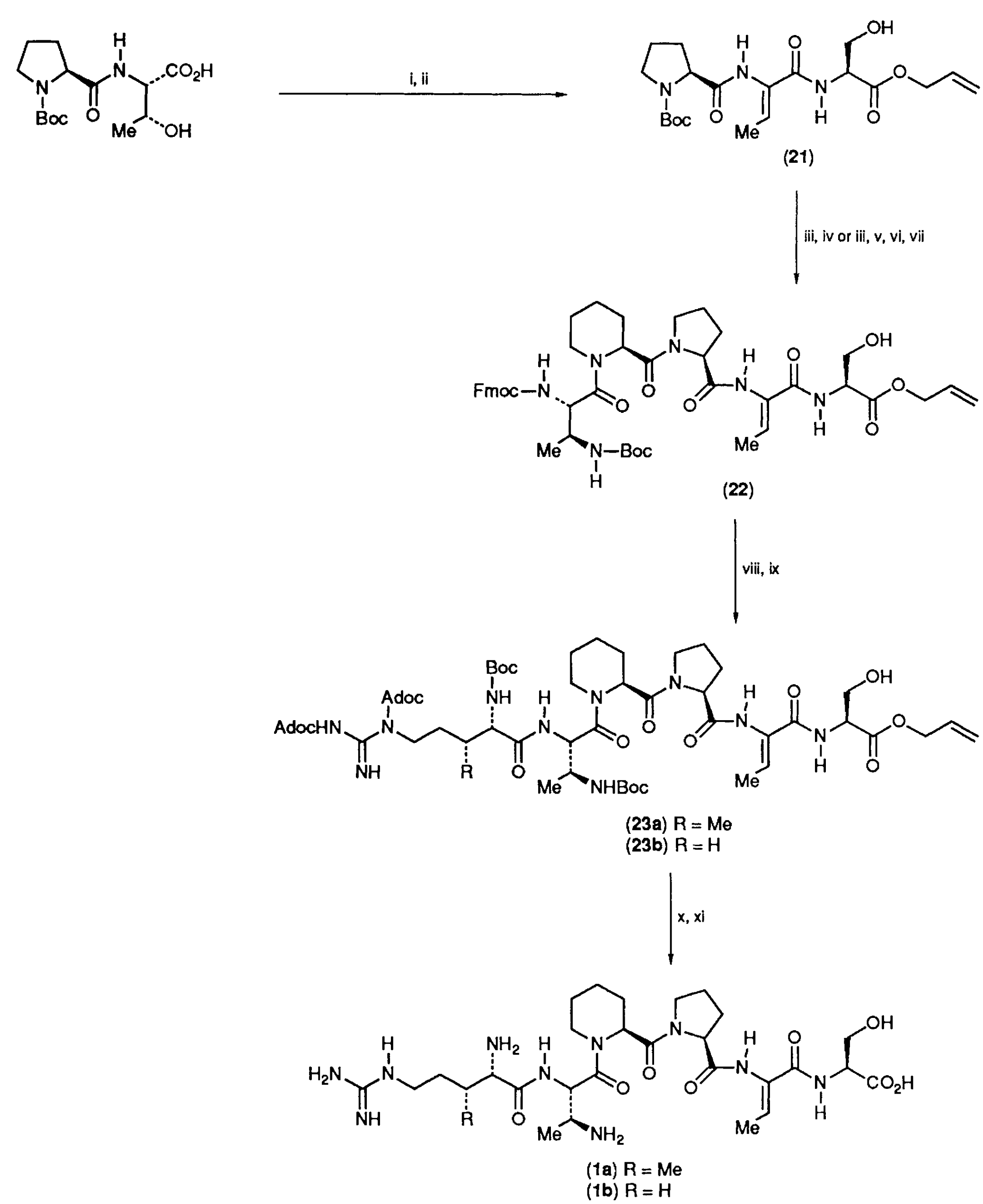
Scheme 2. *Reagents and conditions*: i, $EtO_2C-CH_2-PO-(OEt)_2$, NaH, tetrahydrofuran (THF), room temp., 12 h, 81%, *E*/*Z* 99/1; ii, di-isobutylaluminium hydride (DiBAH), CH_2Cl_2, hexane, −78 °C, 3 h, 94%; iii, L(+)-diethyl tartrate (DET), $Ti(OPr^i)_4$, Bu^tOOH, CH_2Cl_2, −20 °C, 3 h, 72% (98% enantiomeric excess, e.e.); iv, $AlMe_3$, toluene, hexane, room temp., 2 h, 65%, (1,2-diol/1,3-diol = 90/10); v, Z–Cl, pyridine, dimethylaminopyridine (DMAP), CH_2Cl_2, 0 °C to room temp., 8 h, 53%; vi, HN_3, PPh_3, DEAD, toluene, room temp., 2 h, 93%; vii, MeOH, H_2, Pd, room temp., 5 h; viii, $COCl_2$, toluene, KOH, 0 °C, 4 h, vii + viii: 79%; ix, BBr_3, CH_2Cl_2, 20—50 °C, 3 h, 77%; x, NaN_3, dimethylformamide (DMF), 140 °C, 16 h, 84%; xi, $(Boc)_2O$, DMAP, Et_3N, CH_2Cl_2, room temp., 12 h, 87%; xii, $CsCO_3$, CH_3OH, room temp., 14 h, 78%; xiii, pyridinium dichromate (PDC), DMF, room temp., 12 h, 81%; xiv, H_2, Pd, MeOH, room temp., 5 h, 82%; xv, $H_2N-C(NH)-SO_3H$, K_2CO_3, room temp., 14 h, 67%; xvi, Adoc–Cl, NaOH, dioxane 0—20 °C, 12 h, 73%.

amides since threonine esters undergo elimination of water to furnish the didehydroaminobutyric esters in the presence of azodicarboxylates and triphenylphosphine. The (3*R*)-hydroxy group in Fmoc-(2*S*,3*R*)-threonyl-(*S*)-pipecolinic acid trichloroethyl ester (**2**) can be readily exchanged for a (3*S*)-azide group. In order to avoid the possibility of a reduction of the trichloroethyl ester to an ethyl ester, we employed the Lindlar catalyst for the reduction of the azide group to an amine function[10] and then protected the latter as the Boc derivative (**4**). (2*S*)-Fmoc-amino-(3*S*)-Boc-aminobutyric acid (**8**) was obtained in 57% yield by the Mitsunobu reaction of Fmoc-(2*S*,3*R*)-threonine Boc-hydrazide (**6**) followed by hydrolysis, hydrogenation, and acylation.

The configuration of (2*S*,3*R*)-3-methylarginine was predetermined by its unambiguous, diastereoselective construction (Scheme 2). The substituted *trans*-allyl alcohol (**10**) is readily accessible through condensation of ethoxypropionaldehyde with triethyl phosphonoacetate and reduction of the thus-formed unsaturated ester with di-isobutylaluminium hydride. The two stereogenic centres were then constructed by means of Sharpless oxidation.[11] Ring opening of the epoxide (**11**) with trimethylaluminium[12] proceeded with 90% regioselectivity to furnish (**12**). The primary alcohol group was then masked as its carbonate ester while the secondary alcohol group was converted by the Mitsunobu reaction to an azide function, thus yielding (**13**). Catalytic hydrogenation then produced the amino alcohol which was masked as the oxazolidinone (**14**). Boron trifluoride cleavage of the ether gave the bromide (**15**)† which, on reaction with sodium azide, furnished the diamino derivative (**16**) possessing two differently masked nitrogen atoms. After acylation of the nitrogen atom, the oxazolidinone ring could be cleaved easily with caesium carbonate[13] to furnish (**17**). Oxidation of the alcohol group and reduction of the azide group gave rise to the amino acid derivative (**18**). Construction of the guanidine functional group[14] and its protection as the bisadamantyloxycarbonyl derivative[3] were performed using standard procedures. The resultant methylarginine derivative (**20**) was employed in our synthesis of lavendomycin.

First of all, we prepared norlavendomycin (**1b**) (Scheme 3). For the synthesis of the right-hand half of the hexapeptide, Boc-Pro-Thr-OH was allowed to react with 2 mol equiv. of disuccinimidyl carbonate for concomitant elimination of water

† The direct oxidation of 2-Boc-amino-3-methyl-5-ethoxypentanol obtained from (**13**) to the 5-ethoxyisoleucine derivative could be performed easily, but all reactions that cause ether cleavage lead to extensive epimerisation and ring closure to the pyrrolidine derivative.



Scheme 3. *Reagents and conditions*: i, disuccinimidyl carbonate (DSC) (2 mol equiv.), Et_3N, CH_2Cl_2, room temp., 6 h; ii, H-Ser-OAll·HCl, Et_3N, CH_2Cl_2, room temp., 6 h, i + ii: 42%; iii, HCl–dioxane, CH_2Cl_2, 0 °C to room temp., 1 h, quant.; iv, (**5**), benzotriazole-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP), Et_3N, MeCN, 0 °C to room temp., 1 h, 48%; v, Boc-Pip-OH, bis-(2-oxo-oxazolidin-3-yl) phosphinic chloride (BOP-Cl), $EtNPr^i_2$, CH_2Cl_2, 0 °C to room temp., 6 h, 52%; vi, HCl–dioxane, CH_2Cl_2, 0 °C to room temp., 1 h, quant.; vii, (**8**), hydroxybenzotriazole (HOBt), *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride, Et_3N, CH_2Cl_2, −20 °C to room temp., 8 h, 49%; viii, 1,8-diazabicyclo[5.4.0]undec-7-ene (1.5—5 mol equiv.) (DBU), ethyl acetate, 70 °C, 2 h, 95%; ix, Boc(Adoc)$_2$Arg-OH or Boc(Adoc)$_2$MeArg-OH (**20**), BOP, Et_3N, CH_2Cl_2, room temp., 12 h, 41%; x, $Pd(PPh_3)_4$, morpholine, THF, room temp., 1 h, 90%; xi, trifluoroacetic anhydride, CH_2Cl_2, 2 h, Sephadex C25, 79%.

and activation and then with serine allyl ester to furnish the peptide (**21**). Pipecolinic acid and diamino-butyric acid can be added separately or as the respective dipeptide. After deprotection of the ester group in (**4**) with zinc–acetic acid and cleavage of the Boc group from (**21**), coupling yielded the pentapeptide (**22**). Cleavage of the Fmoc protective group and coupling with Boc-(Adoc)$_2$-arginine then furnished the masked norlavendomycin (**23b**) from which the allyl group was removed first, followed by simultaneous cleavage of the two Boc groups and the Adoc groups on treatment with trifluoroacetic acid. Norlavendomycin‡ (**1b**) was separated and purified by chromatography on Sephadex C25 and Diaion.

Lavendomycin (**1a**) was prepared analogously by using the methylarginine derivative (**20**). The product of this synthesis was found to be identical with the natural product in every respect. Noteworthy is the strong influence of the methyl group of the methylarginine on both the basicity and the chromatographic behaviour. Both methylarginine and lavendomycin are more basic than arginine and norlavendomycin, respectively, and are thus eluted considerably more slowly on chromatography over Sephadex C25 and Diaion. We have not employed the 3-epimer of methylarginine and built up the corresponding lavendomycin epimer since the (2*S*,3*R*)-configuration of the methylarginine in naturally occurring lavendomycin is unequivocally confirmed by the excellent agreement of the ^{1}H and ^{13}C NMR spectra of the synthetic and natural products and those of the methylarginine obtained from the hydrolysate of naturally occurring lavendomycin.

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‡ Selected spectroscopic data for (**1b**) and (**23a**): (**1b**): ^{1}H NMR (300 MHz, D_2O): δ 1.24 (d, *J* 6.66 Hz, 3H), 1.73 (d, *J* 7 Hz, 3H), 1.40—2.14 (m, 13H), 2.45 (m, 1H), 3.10—3.30 (m, 3H), 3.60—3.94 (m, 7H), 4.14 (t, *J* 6.5 Hz, 1H), 4.36 (dd, *J* 4.07, 5.22 Hz, 1H), 4.51 (t, *J* 7.41 Hz, 1H), 4.98 (t, *J* 5.43 Hz, 1H), 5.34 (d, *J* 4.46 Hz, 1H), 6.82 (q, *J* 7 Hz, 1H); MS (FAB): $(M + H)^+$: *m/z* 653 (65%), 565 (3), 397 (3), 340 (3)

(**23a**): ^{1}H NMR (300 MHz, $CDCl_3$, internal $SiMe_4$): δ 0.95 (2 d, br, 6H), 1.30—1.50 (m, 19H), 1.52—1.80 (m, 21H), 1.80—2.30 (m, 23H), 2.40 (m, 1H), 3.45—4.10 (m, 10H), 4.30—4.50 (m, 2H), 4.62—4.67 (m, 3H), 4.95—5.20 (m, 3H), 5.12 (dd, *J* 2.0, 7.8 Hz, 1H), 5.21 (dd, *J* 1.23, 10.44 Hz, 1H), 5.33 (dd, *J* 1.47, 17.5 Hz, 1H), 5.58 (d, *J* 8.02 Hz, 1H), 5.9 (m, 1H), 6.81 (q, *J* 7.04 Hz, 1H), 7.05 (d, *J* 7.30 Hz, 1H, 7.39 (d, *J* 7.46 Hz, 1H), 7.77 (s, 1H), 9.05—9.35 (br., 2H); $[\alpha]_D^{20}$ −21.75° (*c* 1.6, $CHCl_3$)

References

1 Amino Acids and Peptides, Part 76. Part 75: U. Schmidt, A. Lieberknecht, U. Kazmaier, H. Griesser, G. Jung, and J. Metzger, *Synthesis*, 1990, in the press.
2 Isolation: T. Komori, M. Ezaki, E. Kino, M. Kohsala, H. Aoki, and H. Imanaka, *J. Antibiot.*, 1985, **38**, 691; structural elucidation: I. Uchida, N. Shigematsu, M. Ezaki, and M. Hashimoto, *Chem. Pharm. Bull.*, 1985, **33**, 3053.
3 G. Jäger and R. Geiger, *Chem. Ber.*, 1970, **103**, 1727.
4 P. P. Jeffrey and S. W. McCombi, *J. Org. Chem.*, 1982, **47**, 587; H. Kunz and H. Waldmann, *Angew. Chem.*, 1984, **96**, 49.
5 L. A. Carpino and G. Y. Han, *J. Am. Chem. Soc.*, 1970, **92**, 5748.
6 R. B. Woodward, K. Hensler, J. Gosteki, P. Naegeli, W. Oppolzer, R. Ramage, S. Ranaganthan, and H. Vorbrüggen, *J. Am. Chem. Soc.*, 1966, **88**, 852.
7 H. Ogura, O. Sato, and K. Takeda, *Tetrahedron Lett.*, 1981, **22**, 4817.
8 E. Atherton and J. Meienhofer, *Hoppe-Seyler's Z. Physiol. Chem.*, 1973, **354**, 689.
9 O. Mitsunobu, *Synthesis*, 1981, 1; H. Loibner and E. Zbiral, *Helv. Chim. Acta*, 1976, **59**, 2100.
10 E. J. Corey, K. C. Nicolaou, R. D. Balanson, and Y. Machida, *Synthesis*, 1975, 590.
11 T. Katsuki and K. B. Sharpless, *J. Am. Chem. Soc.*, 1980, **102**, 5974.
12 T. Suzuki, M. Saimoto, H. Tomioka, and K. Ohisma, *Tetrahedron Lett.*, 1982, **23**, 3597.
13 T. Ishizuka and T. Kuneida, *Tetrahedron Lett.*, 1987, **28**, 4185.
14 K. Kim, Y. T. Lin, and M. S. Mosher, *Tetrahedron Lett.*, 1988, **29**, 3183.