Convenient Synthesis of the Epoxy Fragment of Azinomycin B

Yaeko KONDA,* Tomomi MACHIDA, Tomomitsu SASAKI, Kazuyoshi TAKEDA, Hiroaki TAKAYANAGI, and Yoshihiro HARIGAYA

School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, Japan.
Received July 21, 1993; accepted September 10, 1993

A new convenient route for asymmetric synthesis of the epoxy fragment of azinomycin B by Sharpless asymmetric epoxidation of secondary allylic alcohol is described.

Keywords azinomycin B; Sharpless asymmetric epoxidation; allylic alcohol; asymmetric synthesis; epoxide; X-ray

Azinomycins A (**1a**) and B (**1b**) are antitumor antibiotics isolated by Nagaoka *et al.*[1a,c] in 1986, and their structures were determined by nuclear magnetic resonance (NMR) studies.[1b] We have examined the structure of carzinophilin (CZP) (**2**), isolated from *Streptomyces sahachiroi* by Hata *et al.*[2] in 1954, and we found that CZP is identical[3] with azinomycin B by detailed comparison of ^{1}H- and ^{13}C-NMR and reinvestigation of the FAB mass spectrum (FAB-MS).[3] In 1991, Armstrong *et al.* also reported the identity of these two compounds.[4,5]

Azinomycin B is an interesting compound having a very unusual type of structure not related to any other natural products and it shows potent antitumor activity, comparable to that of mitomycin C.[6] However, the structure has only been determined by means of NMR analysis and not been precisely elucidated by X-ray analysis or confirmed by synthesis. So we started synthetic studies several years ago. The synthesis of the epoxy fragment was first reported by Ando *et al.*[7] from fructose as a starting material. In this paper, we wish to report a convenient asymmetric synthesis of the epoxy fragment **5a** using kinetic resolution in Sharpless asymmetric epoxidation of a racemic allylic alcohol, benzyl 2-hydroxy-3-methyl-3-butenoate (**4**).

Armstrong *et al.* have recently synthesized[5] a derivative of the epoxy fragment **5a** by a kinetic resolution in Sharpless asymmetric epoxidation of a racemic allylic alcohol, the 4-methoxybenzyl ether of 2-hydroxy-3-methyl-3-butene-1-ol, and after that, Shibuya *et al.* reported[8] another synthetic method for a derivative of **5a** by Sharpless asymmetric epoxidation of prochiral diisopropenyl carbinol, during the course of our work. However, our method differs from their routes in the substrate.

The epoxy fragment should have 2*S*,3*S*-configuration on the basis of our report[9] and several reports by other workers.[5,8,10] The desired compound **5a** was easily obtained asymmetrically (73% enantiomeric excess (ee)) in four steps from acetone as follows.

Transesterification of **3**, which was easily prepared by a known method from acetone in two steps,[11,12] with benzyl alcohol afforded the corresponding benzyl ester **4** (56%).

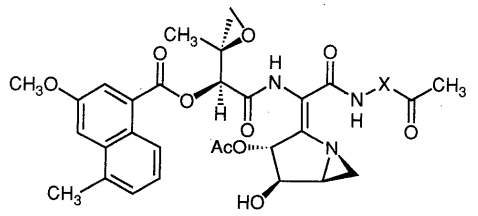
Sharpless asymmetric epoxidation of racemic allylic alcohol **4** with (−)-diethyl tartrate (DETA) and 2 eq of *tert*-butyl hydroperoxide (TBHP) gave the 2*R*,3*R*-*erythro*-epoxy alcohol **5b** (38%, 46% ee) and the 2*S* allylic alcohol **4a** (38%). In Sharpless epoxidation of the racemic *sec*-allylic alcohol, it has been reported that the resulting epoxy alcohol usually has *erythro* form[13a] predominantly.

It is also expected that the allylic alcohol **4** should afford the 2*S*,3*S*-*erythro*-epoxy alcohol **5a** from a consideration of the usual enantiofacial selectivity[13b] of the resulting epoxide ring in the case of using (−)-DETA. However, we unexpectedly obtained the undesired 2*R*,3*R*-*erythro* isomer **5b**.

On the contrary, Sharpless asymmetric epoxidation of **4** with (+)-DETA and 0.6 eq of TBHP gave the desired 2*S*,3*S*-*erythro*-epoxy alcohol **5a** (35%, 73% ee) and recovered 2*R* allylic alcohol **4b** (45%). When 2 eq of TBHP was used, the enantioselectivity of **5a** was decreased (35%, 43% ee). These reactions are considered to be diastereoselective because none of the *threo*-epoxy alcohol (2*S*,3*R* or 2*R*,3*S*) was obtained from the reaction mixture.

Absolute configurations of **5a** and **5b** were deduced as follows. Comparison of the ^{1}H-NMR spectra and optical rotation of **5a** with the published data[7] for the 2*S*,3*S*-*erythro*-epoxy alcohol indicated that **5a** has the same configuration, 2*S*,3*S*-*erythro* form, and consequently **5b** has 2*R*,3*R*-*erythro* form.

In addition, the configuration of C-2 in **5a** and **5b** was determined chemically as follows. On Sharpless asymmetric epoxidation of the racemic secondary allylic alcohol **4** in the presence of (−)- and (+)-DETA, the less reactive chiral allylic alcohol (**4a** and **4b** respectively) was recovered. Hydrogenation of **4a** and **4b** over Pd–C gave known chiral 2-hydroxy-3-methylbutanoic acids **6a** and **6b**, respectively. The carboxylic acid **6a** exhibited positive specific rotation, and **6b** had negative one. It was reported[14] that (*S*)- and (*R*)-2-hydroxy-3-methylbutanoic acids have positive and negative specific rotation, respec-



1a: azinomycin A X = CH_2
1b: azinomycin B X = C=CH(OH) (carzinophilin) (**2**)

Chart 1

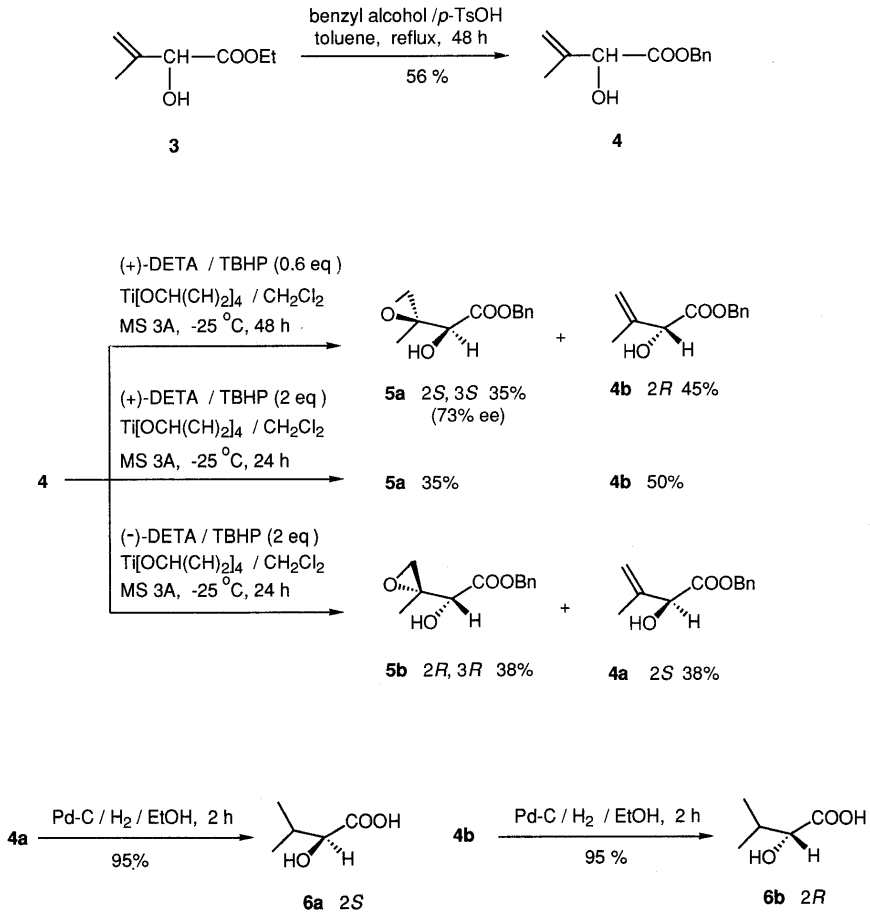


Chart 2

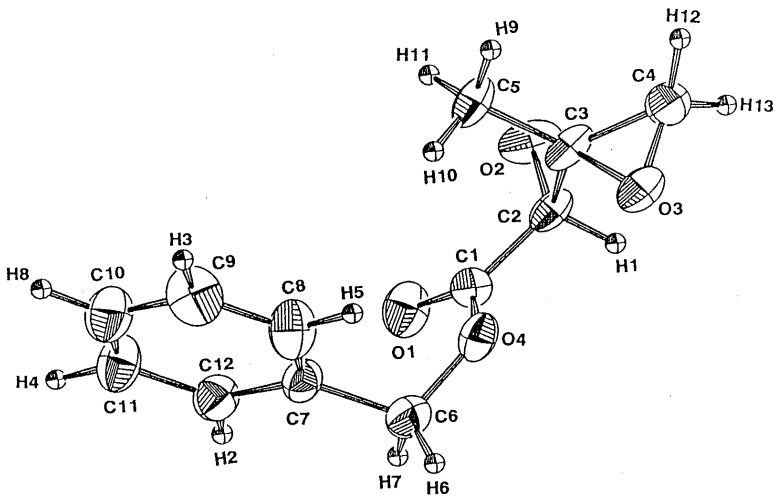


Fig. 1

tively. Thus, *S*-**6a** and *R*-**6b** were confirmed. Accordingly, **4a** and **4b** have 2*S* and 2*R*-configuration, respectively. Consequently, the absolute configuration of the *erythro*-epoxy alcohols **5a**, **5b** were definitively determined as 2*S*,3*S* and 2*R*,3*R* respectively.

Finally, we examined X-ray analysis of **5a**. An ORTEP view of **5a** exhibited the *erythro* form as we deduced.

In conclusion, the epoxy fragment of azinomycin B was conveniently synthesized asymmetrically (73% ee) by a new route in four steps from acetone, though it is necessary to separate enantiomers by an analytical method such as HPLC to obtain an enantiomerically pure product. It is noteworthy that Sharpless asymmetric epoxidation of racemic benzyl 2-hydroxy-3-methyl-3-butenoate **4** showed an unusual enantiofacial selectivity.

Experimental

Melting points were measured on a micro hot-stage apparatus and

are uncorrected. Optical rotations were taken on a JASCO model DPI −181 polarimeter. The infrared (IR) spectrum was recorded on a Hitachi 260-30 spectrometer. ^{1}H-NMR spectra were taken on a Varian VXR-300 or XL-400 spectrometer in deuteriochloroform unless otherwise stated, and high resolution (HR)-FAB-MS and field desorption (FD)-MS on a JEOL JMS-DX-300. Chemical yield was calculated on the basis of the weights of starting materials. ee was determined by NMR analysis using a chiral shift reagent, (Eu(hfc)$_3$) (c=0.66—0.93; c=molar ratio : shift reagent/substrate).

Benzyl 2-Hydroxy-3-methyl-3-butenoate (4) *p*-Toluenesulfonic acid (200 mg, 1.161 mmol) was added to a solution of ethyl 2-hydroxy-3-methyl-3-butenoate (**3**)[11,12] (2 g, 0.014 mol) and benzyl alcohol (6 ml, 0.056 mol) in toluene (15 ml), and the mixture was refluxed for 45 h, then evaporated to dryness. Flash column chromatography (silica gel, *n*-hexane : $CHCl_3$ = 10 : 3) of the residue afforded **4** as a colorless oil (56.4%). HR-FAB-MS *m/z*: Calcd for $C_{12}H_{14}O_3$+Na: 229.0841. Found: 229.0841. ^{1}H-NMR: δ 7.34 (s, aromatic-H$_5$), 5.21 (s, OCH$_2$), 5.10, 4.96 (each d, J=1.5 Hz, 4-H$_2$), 4.58 (d, J=5.7 Hz, 2-H), 3.03 (d, J=5.7 Hz, 2-OH) 1.69 (s, 3-CH$_3$).

Benzyl (2*S*,3*S*)-2-Hydroxy-3-methyl-3,4-epoxybutanoate (5a) Titanium(IV) isopropoxide (16.5 mg, 0.15 eq) and **4** (80 mg, 0.39 mmol) were added to a solution of (+)-DETA (12 mg, 0.15 eq) in CH_2Cl_2 (8 ml) in the presence of 3A molecular sieves (50 mg), and the resulting mixture was stirred at −20 °C for 30 min. Then 25% TBHP solution (toluene : CH_2Cl_2 = 1 : 1, 0.35 ml, 0.6 eq) was added, and the reaction was continued for 48 h. Dimethyl sulfide (0.11 ml, 1.56 mmol) was next added. The reaction mixture was stirred for 40 min at −20 °C, and saturated NaF solution (2 ml) and an appropriate amount of NaCl were added at room temperature under stirring. The precipitate was removed by filtration through Celite and the phases were separated. The aqueous layer was extracted with CH_2Cl_2 (10 ml × 2) and the combined organic extracts were concentrated to dryness. Preparative TLC (silica gel, $CHCl_3$: MeOH = 50 : 1) of the residue gave the epoxy alcohol **5a** as a colorless oil (38 mg, 35%, 73% ee) and recovered chiral allylic alcohol **4b** as a colorless oil (50 mg, 45%). **5a** was crystallized from $CHCl_3$ in a refrigerator as colorless plates.

5a: mp 32—33 °C. $[\alpha]_D^{22}$ −9.37° (c=1.90, $CHCl_3$) (lit.[7] −22.4°, EtOH). HR-FAB-MS *m/z*: Calcd for $C_{12}H_{14}O_4$+Na: 245.0790. Found: 245.0789. ^{1}H-NMR: δ 7.42—7.32 (m, aromatic-H$_5$), 5.32, 5.26 (each d, J=12.0 Hz, OCH$_2$), 4.00 (d, J=5.0 Hz, 2-H), 2.98 (d, J=5.0 Hz, 2-OH), 2.86, 2.65 (each d, J=4.5 Hz, 4-H$_2$), 1.31 (s, 3-CH$_3$). The ^{1}H-NMR spectrum of **5a** was in accordance with the reported data.[7]

4b: $[\alpha]_D^{22}$ +38.4° (c=0.45, $CHCl_3$). HR-FAB-MS *m/z*: Calcd for $C_{12}H_{14}O_3$+Na: 229.0841. Found: 229.0865. The ^{1}H-NMR spectrum of **4b** was identical with that of the racemic allyl alcohol **4**.

By using a procedure analogous to that described for **5a** with TBHP (2 eq) for 24 h, **5a** (35.3%, 43% ee) and **4b** (50%) were obtained, each as a colorless oil.

5a: $[\alpha]_D^{22}$ −8.27° (c=1.50, EtOH). **4b**: $[\alpha]_D^{22}$ +19.9° (c=3.10, EtOH). The ^{1}H-NMR spectra of **5a** and **4b** were in accordance with the above data.

Benzyl (2*R*,3*R*)-2-Hydroxy-3-methyl-3,4-epoxybutanoate (5b) By using a procedure analogous to that described for **5a** with (−)-DETA and TBHP (2 eq) for 24 h, **5b** (38%, 46% ee) and **4a** (38%) were obtained, each as a colorless oil. The ^{1}H-NMR spectra of **5b** and **4a** were identical

TABLE I. Positional Parameters and B_{eq} for **5a**

Atom	x	y	z	B_{eq}
C(1)	0.636 (1)	0.6758	0.6605 (7)	4.0 (2)
O(4)	0.5693 (6)	0.485 (2)	0.6819 (5)	4.7 (2)
C(2)	0.6971 (9)	0.661 (2)	0.5580 (7)	3.9 (3)
O(2)	0.8033 (7)	0.838 (2)	0.5515 (6)	5.7 (3)
C(3)	0.795 (1)	0.454 (2)	0.5511 (8)	4.2 (3)
O(1)	0.6380 (7)	0.838 (2)	0.7213 (5)	5.9 (3)
C(7)	0.632 (1)	0.453 (2)	0.8970 (7)	4.4 (4)
C(12)	0.655 (1)	0.616 (2)	0.9810 (8)	5.1 (4)
C(9)	0.855 (1)	0.245 (2)	1.032 (1)	6.8 (5)
C(11)	0.780 (1)	0.591 (2)	1.0910 (8)	6.2 (5)
C(8)	0.731 (1)	0.267 (2)	0.9217 (8)	6.1 (5)
C(6)	0.498 (1)	0.476 (3)	0.7770 (8)	6.1 (5)
C(10)	0.877 (1)	0.413 (2)	1.1152 (9)	6.2 (5)
O(3)	0.6940 (6)	0.268 (2)	0.4957 (5)	4.5 (2)
C(5)	0.9371 (9)	0.398 (2)	0.6616 (7)	4.7 (3)
C(4)	0.797 (1)	0.375 (2)	0.4389 (7)	4.7 (4)
H(1)	0.6011	0.6681	0.4874	4.6
H(2)	0.5860	0.7486	0.9651	6.4
H(3)	0.9286	0.1132	1.0549	8.1
H(4)	0.7944	0.7016	1.1536	7.3
H(5)	0.7211	0.1515	0.8628	7.9
H(6)	0.4170	0.3609	0.7674	7.4
H(7)	0.4356	0.6149	0.7801	7.4
H(8)	0.9664	0.4016	1.1909	7.4
H(9)	1.0117	0.2912	0.6451	6.1
H(10)	0.8954	0.3364	0.7222	6.1
H(11)	1.0006	0.5263	0.6939	6.1
H(12)	0.8924	0.3000	0.4236	5.5
H(13)	0.7474	0.4589	0.3628	5.5

TABLE II. Bond Lengths (Å) of **5a**

Atom	Distance	Atom	Distance
C1–O4	1.344 (9)	C9–C8	1.41 (1)
C1–C2	1.45 (1)	C9–C10	1.39 (1)
C1–O1	1.216 (9)	C9–H3	0.999
O4–C6	1.426 (9)	C11–C10	1.33 (1)
C2–O2	1.416 (9)	C11–H4	0.977
C2–C3	1.52 (1)	C8–H5	0.972
C2–H1	0.971	C6–H6	0.962
C3–O3	1.444 (9)	C6–H7	0.999
C3–C5	1.52 (1)	C10–H8	0.981
C3–C4	1.41 (1)	O3–C4	1.405 (9)
C7–C12	1.37 (1)	C5–H9	0.963
C7–C8	1.39 (1)	C5–H10	0.963
C7–C6	1.53 (1)	C5–H11	0.956
C12–C11	1.41 (1)	C4–H12	0.991
C12–H2	0.978	C4–H13	1.006

Distances are in angstroms. Estimated standard deviations in the least significant figure are given in parentheses.

TABLE III. Bond Angles (°) of **5a**

Atom	Angle	Atom	Angle
O4–C1–C2	112.2 (7)	C12–C11–H4	120.58
O4–C1–O1	121.1 (7)	C10–C11–H4	117.94
C2–C1–O1	126.6 (8)	C7–C8–C9	120.2 (9)
C1–O4–C6	118.7 (7)	C7–C8–H5	121.29
C1–C2–O2	112.8 (7)	C9–C8–H5	118.47
C1–C2–C3	115.4 (7)	O4–C6–C7	111.5 (6)
C1–C2–H1	107.39	O4–C6–H6	112.86
O2–C2–C3	105.2 (6)	O4–C6–H7	110.54
O2–C2–H1	107.28	C7–C6–H6	109.85
C3–C2–H1	108.31	C7–C6–H7	107.10
C2–C3–O3	114.5 (6)	H6–C6–H7	104.58
C2–C3–C5	116.0 (7)	C9–C10–C11	120.2 (9)
C2–C3–C4	119.3 (7)	C9–C10–H8	119.72
O3–C3–C5	114.7 (6)	C11–C10–H8	120.08
O3–C3–C4	58.9 (5)	C3–O3–C4	59.4 (5)
C5–C3–C4	120.1 (7)	C3–C5–H9	111.69
C12–C7–C8	119.4 (8)	C3–C5–H10	110.74
C12–C7–C6	120.5 (9)	C3–C5–H11	111.14
C8–C7–C6	120.1 (9)	H9–C5–H10	107.35
C7–C12–C11	119.5 (9)	H9–C5–H11	107.89
C7–C12–H2	120.49	H10–C5–H11	107.85
C11–C12–H2	119.99	C3–C4–O3	61.7 (5)
C8–C9–C10	119 (1)	C3–C4–H12	124.55
C8–C9–H3	123.38	C3–C4–H13	123.31
C10–C9–H3	117.22	O3–C4–H12	121.93
C12–C11–C10	121 (1)	O3–C4–H13	120.94
		H12–C4–H13	101.92

Angles are in degrees. Estimated standard deviations in the least significant figure are given in parentheses.

with those of **5a** and **4**, respectively.

5b: $[\alpha]_D^{22}$ +13.09° (c=1.00, $CHCl_3$). HR-FAB-MS m/z: Calcd for $C_{12}H_{14}O_4$+Na: 245.0790. Found: 245.0793. **4a**: $[\alpha]_D^{22}$ −40.38° (c=1.00, $CHCl_3$).

(2*S*)-2-Hydroxy-3-methylbutanoic Acid (6a) A solution of **4a** (15 mg) in ethanol (5 ml) was hydrogenated over H_2/Pd–C (10.0 mg) for 1 h. After work-up as usual, the reaction mixture afforded **6a** as an oily compound (7.5 mg, 95%).

6a: $[\alpha]_D^{22}$ +10.88° (c=0.57, MeOH) (lit.[14] +19.0°, $CHCl_3$). FD-MS m/z: M^++H, 119. ^{1}H-NMR (CD_3OD): δ 3.92 (d, J=4.0 Hz, 2-H), 2.05 (d quint., J=7.0, 4.0 Hz, 3-H), 1.00, 0.91 (each d, J=7.0 Hz, $CH_3\times2$).

(2*R*)-2-Hydroxy-3-methylbutanoic Acid (6b) A solution of **4b** (9 mg) in ethanol (5 ml) was hydrogenated over H_2/Pd–C (7 mg) for 1 h. After work-up, **6b** was obtained as colorless crystals (4.9 mg, 95.0%).

6b: mp 55—56 °C. $[\alpha]_D^{22}$ −8.67° (c=0.30, EtOH) (lit.[14] −20.0°, $CHCl_3$). The ^{1}H-NMR spectrum was identical with that of **6a**.

X-Ray Crystallographic Study of 5a $C_{12}H_{14}O_4$, M_r=222.24, colorless plates, crystal size 0.40×0.40×0.40 mm, monoclinic, space group $P2_1$ (No. 8), a=8.433(4) Å, b=6.067(4) Å, c=11.808(4) Å, β=107.68(3) Å, V=575.6(5) Å^3, Z=2, D_{calcd}=1.282 g·cm^{-3}, $\mu(CuK_\alpha)$=7.61 cm^{-1}. Intensity data were collected at room temperature with graphite monochromated CuK_α radiation (λ=1.54178 Å) on a Rigaku AFC-5R diffractometer: $2\theta_{max}$=140.3°. Of 1242 measured reflections, 888 had $I>3\sigma(I)$. The structure was solved by direct methods and refined to R=0.076 and R_w=0.075 using the Texan-Textray Structure Analysis Package from Molecular Structure Corporation (1985). The final positional and thermal parameters, bond distances, and angles are presented in Tables I, II and III, respectively. The authors have deposited the atomic coordinates for this structure with Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from The Director, Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

References and Notes

1) *a*) K. Nagaoka, M. Matsumoto, J. Oono, K. Yokoi, S. Ishizaki, T. Nakashima, *J. Antibiotics*, **39**, 1527 (1986); *b*) K. Yokoi, K. Nagaoka, T. Nakashima, *Chem. Pharm. Bull.*, **34**, 4554 (1986); *c*) S. Ishizaki, M. Ohtsuka, K. Kukita, K. Nagaoka, T. Nakashima, *J. Antibiotics*, **40**, 60 (1987).
2) T. Hata, F. Koga, Y. Sano, K. Kanamori, A. Matsumae, R. Sugawara, T. Shima, S. Ito, S. Tomozawa, *J. Antibiotics Ser. A*, **7**, 107 (1954).
3) Unpublished results. ^{1}H- and ^{13}C-NMR data of carzinophilin and azinomycin B (400 MHz) are superimposable. HR-MS m/z: Calcd for $C_{31}H_{34}N_{30}O_{11}$: 624.2208. Found: 624.2209.
4) R. W. Armstrong, J. E. Tellew, E. J. Moran, *J. Org. Chem.*, **57**, 2208 (1992).
5) P. England, K. H. Chun, E. J. Moran, R. W. Armstrong, *Tetrahedron Lett.*, **31**, 2669 (1990).
6) *a*) N. Shimada, M. Uekusa, T. Denda, Y. Ishii, T. Iizuka, Y. Sato, T. Hatori, M. Fukui, M. Sudo, *J. Antibiotics Ser. A*, **8**, 67 (1955); *b*) A. Terawaki, J. Greenberg, *Nature* (London), **209**, 481 (1966).
7) K. Ando, T. Yamada, M. Shibuya, *Heterocycles*, **29**, 2209 (1989).
8) K. Shishido, T. Omodani, M. Shibuya, *J. Chem. Soc., Perkin Trans. 1*, **1992**, 2053.
9) M. Onda, Y. Konda, S. Omura, T. Hata, *Chem. Pharm. Bull.*, **19**, 2013 (1971).
10) M. Shibuya, H. Terauchi, *Tetrahedron Lett.*, **28**, 2619 (1987).
11) P. Yates, J. H. Hoare, *Can. J. Chem.*, **61**, 519 (1983).
12) P. Yates, J. H. Hoare, *Can. J. Chem.*, **61**, 1397 (1983).
13) *a*) V. S. Martin, S. S. Woodard, T. Kasuki, Y. Yamada, M. Ikeda, K. B. Sharpless, *J. Am. Chem. Soc.*, **103**, 6237 (1981); *b*) T. Katsuki, K. B. Sharpless, *ibid.*, **102**, 5976 (1980).
14) P. Koch, Y. Nakatani, B. Luu, G. Ourisson, *Bull. Soc. Chim. Fr.*, **1983**, 189; *idem*, *ibid.*, **1981**, 4723.