SYNTHESIS AND CYTOTOXICITY OF 5-CARBO ANALOGUES OF ACETOMYCIN ‡

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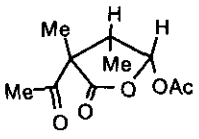
Abstract - Six carbo analogues of anti-cancer antibiotic, acetomycin (**1**) are synthesized. (±)-(3*S**,4*S**,5*R**)-Dihydro-3-acetyl-5-iodomethyl-3,4-dimethyl-2(3*H*)-furanone (**3**α), (±)-(3*S**,4*S**,5*S**)-dihydro-3-acetyl-3,4,5-trimethyl-2(3*H*)-furanone (**4**α), (±)-(3*S**,4*S**,5*R**)-dihydro-3-acetyl-3,4-dimethyl-5-(phenylthio)methyl-2(3*H*)-furanone (**5**α), and their diastereoisomers at C-5, **3**β, **4**β, and **5**β are derived from the same intermediate, (±)-(2*S**,3*R**)-2-(4-methoxybenzyl)oxymethyl-2,3-dimethyl-4-pentenoic acid (**7**) in 5 and 6 steps, respectively. The carbo analogues exhibit perfect resistance in hydrolysis against pig liver esterase. Among them, β-isomers at C-5 indicate more potent inhibition than α-isomers against three tumor cell lines.

Acetomycin (**1**) was isolated from *Streptomyces ramulosus sp.* in 1958 by Prelog *et al.*,[1] and the secondary dimensional structure,[2] and biosynthetic pathway [3] were studied by the same group. Later, the relative and absolute stereochemistries were determined by the X-ray crystallographic analysis for the brominated

‡ Dedicated to Professor Shigeru Oae on the occasion of his 77th birthday.

derivative[4] and acetomycin itself.[5] Initially, acetomycin was reported to possess weak antibacterial activities[1,4] and anti-HSV activities.[6] More recently, however, Mamber *et al.* revealed that it has potential and unique cytotoxic activity *in vitro* assay, against L-1210 murine leukemia cells, and HCT-8 human colon adenocarcinoma cells.[7] Since then, **1** has been the preferred synthetic target molecule for organic chemists, the total syntheses being reported by four groups including the authors.[8] Although acetomycin is a relatively small molecule ($C_{10}H_{14}O_5$, M W 214), its structure is highly oxygenated and has four substituent groups placed consecutively on a small γ-lactone ring. Structure and activity relationships for the complicated three chiral centers including quaternary carbon, however, have yet to be studied well. [9]



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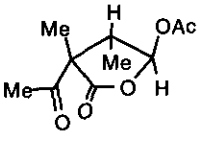

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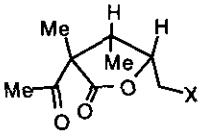
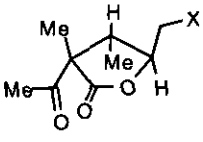


3α X : I
4α X : H
5α X : SPh



3β X : I
4β X : H
5β X : SPh

Acetomycin possesses an attractive antitumor activity, however, it also has a critical disadvantage, its potent activity disappearing *in vivo* system. This inactivation process is due to a rapid hydrolysis of the acetoxy group at C-5 by esterase presented in tissues. [10] Some efforts to synthesize acetomycin derivatives have been made,[11] however, to date the crucial weak point has yet to be overcome. The authors here have prepared several acetomycin derivatives bearing a bulky acyloxy group at C-5 carbon instead of the acetoxy group, which was assumed to be a target part for esterase. They were expected to be inactive against esterase due to the bulkiness, but somehow it was unsuccessful. An alternative idea is that the acyloxy group may be replaced by a carbon substituent group, which might resist against hydrolysis by esterase. In this paper, a synthesis of 5-carbo substituted analogues of acetomycin (**3~5**), and their esterase resistant properties and cytotoxicities against three tumor cell lines are reported.

The syntheses of **3~5** started from carboxylic acid (**7**), which was derived in 6 steps from the important intermediate (**6**) in our total synthesis of acetomycin.[8b] Iodolactonization of **7** by iodine in the presence of

potassium carbonate gave a diastereomeric mixture of **8α** and **8β** in 89% yield. The ratio of the diastereoisomers was identified to be 2.2 : 1 by proton nmr. It was quite difficult to perform these separations by gravinity or flush chromatography, but a small amount of them could be separated by hplc. NOe experiments for the isomers revealed the relative stereochemistries, thus, 15% enhancement was observed between the protons on C-4 and C-5 in the major isomer (**8α**), while less than 2% enhancement was found between the corresponding protons in the minor isomer (**8β**). The proton at C-4 in **8α** appeared at δ 2.49 ppm characteristically as a double quartet with coupling constants of 9.5 and 7.0 Hz. On the other hand, the corresponding proton for (**8β**) appeared at δ 2.05 ppm as a double quartet with coupling constants of 7.3 and 7.0 Hz. As the higher field chemical shift and larger vicinal coupling constant for C-4 proton in the 4,5-*trans* disubstituted compounds have been observed than those in the 4,5-*cis* disubstituted compounds ,[8,9] the above chemical shifts and coupling constants in **8α** and **8β** supported their relative structures.

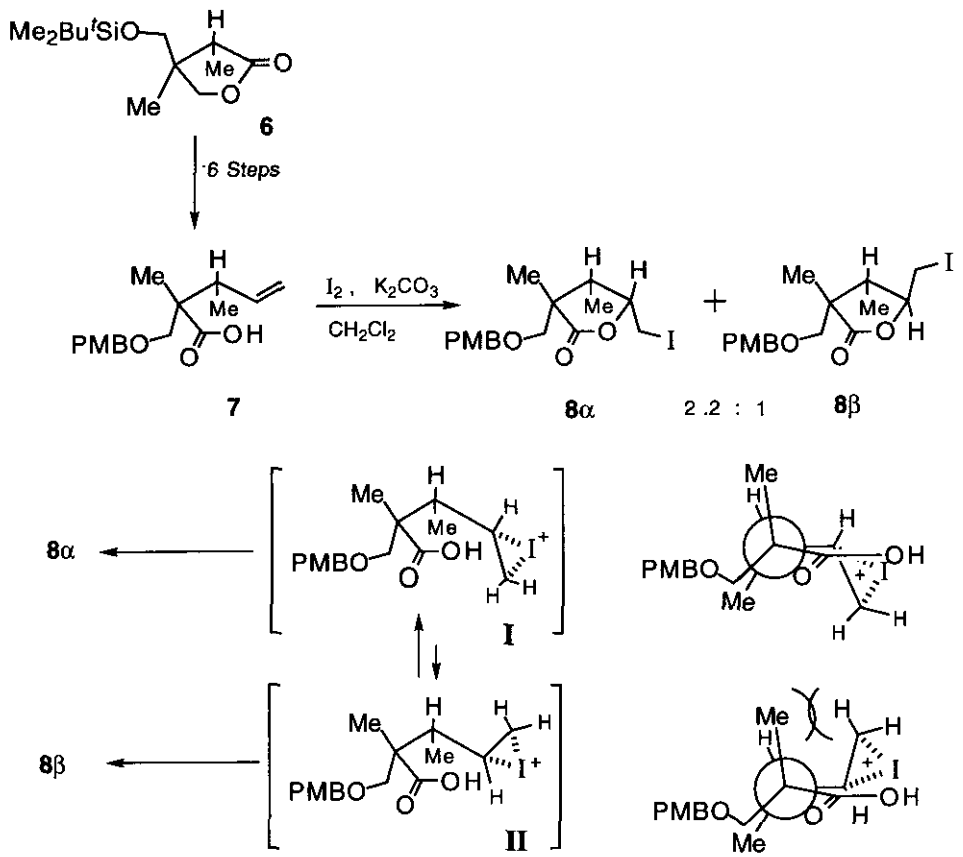
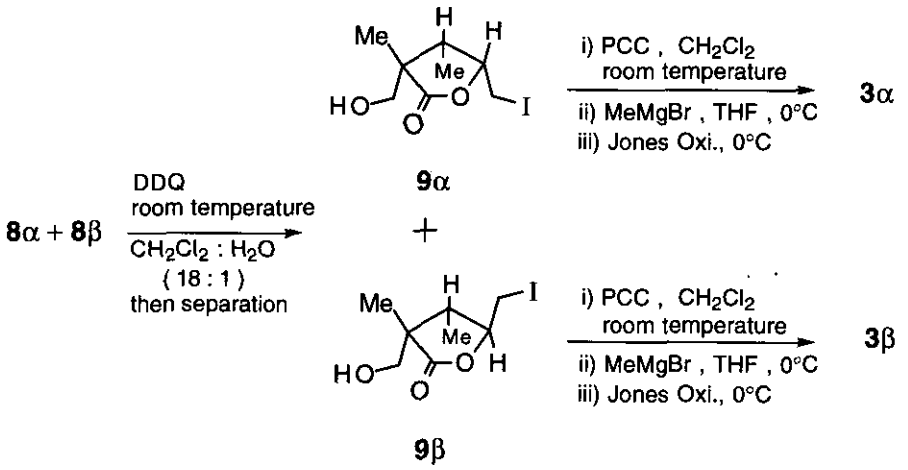


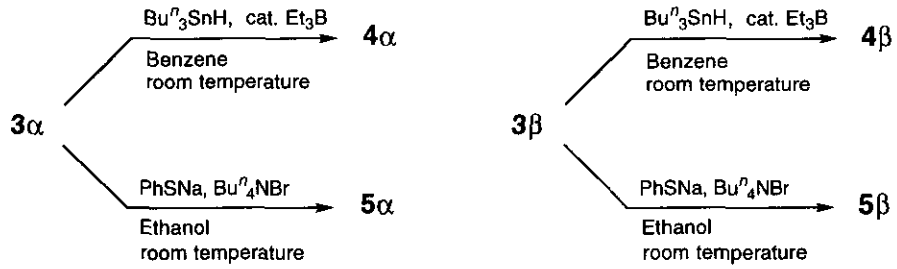
Figure 1. Intermediates leading to **8α** and **8β**

In the cyclization reaction, **8α** was unexpectedly the preferred formation. The newly formed iodomethyl group stood in a sterically unfavorable position on the same α side of (p-methoxybenzyl)oxymethyl and methy groups consecutively on γ-butyrolactone ring. Therefore, this stereoisomer would be expected to have a disadvantage due to a steric hindrance from gauche interactions between the bonds. Here the structures of two intermediates were considered, as illustrated in Figure 1. As the larger (p-methoxybenzyl)oxymethyl group takes an equatorial like position, intermediate (**II**) leading to **8β** would be expected to have a serious 1,3-type steric repulsion between the methyl group and the three-membered iodonium group. On the other hand, intermediate (**I**) leading to **8α** does not have such a steric hindrance between these two groups. By this reasoning, it can be assumed that the cyclization occurred to give **8α** through **I**, predominantly.



Deprotection of the *p*-methoxybenzyl (PMB) group in the mixture of **8α** and **8β** followed by separation using hplc gave **9α** in 65% yield and **9β** in 29% yield. Oxidation of **9α** with PCC afforded aldehyde in 80~95% yield, which was then used for the next Grignard reaction after passing it through Florisil column chromatography. The aldehyde was treated with methylmagnesium bromide at 0°C giving the secondary alcohol. Without purification, the crude alcohol was subjected to Jones oxidation leading to the methyl ketone (**3α**) in 47% yield in three steps. The other stereoisomer (**8β**) was transformed by exactly the same reaction sequence to give **3β** in 39% yield. The methyl derivatives, (**4α** and **4β**), were obtained by reduction of iodides (**3α** and **3β**), respectively. Reduction was carried out in benzene by treating **3α** with tributyltin

hydride in the presence of a catalytic amount of triethylborane at room temperature. The reaction was completed within 5 min to give **4α** in 95% yield. The other isomer (**4β**) was also obtained from **3β** in 92% yield under the same conditions. Substitution of the iodo group in **3α** and **3β** by a phenylsulfenyl group was achieved by a reaction with thiophenol. Iodide (**3α**) was treated with sodium salt of thiophenol in the presence of a catalytic amount of tetrabutylammonium bromide in ethanol at room temperature to provide **5α** in 73% yield. Similarly **3β** afforded **5β** in 97% yield by the same method used for **5α**.



Biological Activity The six compounds (**3α**, **3β** , **4α** , **4β**, **5α**, and **5β**) were subjected to a hydrolysis test by PLE (porcine liver esterase). Each compound was incubated in the presence of PLE in a phosphate buffer solution at pH 8.0. Hydrolysis was checked by tlc and hplc. Although the half life time for acetomycin was found to be 1 h, the 5-carbo analogues prepared above survived completely under the same conditions even after a few days. These results indicated that they are totally inactive against PLE and the 5-carbo derivatives represent the esterase resistancy perfectly. Cytotoxicity was also examined for these compounds, and the results for L-1210, P-388 and KB-cells are shown in Table 1. Although the cytotoxicity of the 5-carbo analogues was less active than that of acetomycin, β-methyl derivative (**4β**) was the most potent among them, having an inhibition of 25 μg/mL level at IC50 against KB cell. It is interesting to note that β-isomers indicated relatively high inhibition. The cytotoxicity of acetomycin 5-epi (5-β-acetoxy) analogue was 10 times weaker than that of acetomycin. This reverse nature of the stereochemistry and cytotoxicity between acetomycin and the 5-carbo analogues was quite surprising. In conclusion, the 5-carbo analogues possess perfect esterase resistant properties but their cytotoxicities are reduced considerably to parent acetomycin.

Table 1. Cytotoxicity of Six 5-Carbo Substituted 5-Desacetoxy Acetomycines (**3α ~ 5β**)

	IC50 (μg/mL)		
Substrate	L-1210	P-388	KB-Cell
3α	>100	>100	>100
4α	35	53	>100
5α	12	28	78
3β	49	7	>100
4β	6	14	25
5β	10	13	80
Acetomycin	0.045	0.52	0.28

EXPERIMENTAL SECTION

Melting points were taken on a Yanako micromelting apparatus and are uncorrected. ^{1}H and ^{13}C nmr were recorded on a Brucker AMX400 and Varian Gemini 300 for ^{1}H (400 MHz or 300 MHz) and for ^{13}C (100 MHz or 75 MHz). The chemical shifts are shown as δ-value (ppm) using tetramethylsilane (0 ppm) for proton spectra and $CHCl_3$ (77.0 ppm) for carbon spectra as an internal standard. Infrared spectra (ir) were recorded by a JASCO RT/IR 230 spectrophotometer and were taken as KBr tablets or film. Low and high resolution mass spectra (LRms and HRms) were obtained on a JEOL JMS 303HF spectrometer by the electron impact (EI) method at 70 eV unless otherwise stated at the Analytical Center in Okayama University of Science. Only significant peaks were described here for ir and mass spectra. Silica gel (Merck 7734, 70-300 mesh) was used for gravity column chromatography and silica gel (Merck 9385, 230-400 mesh) for flash column chromatography. Precoated silica gel plates (Merck 5715, 60F254) were used for thin layer chromatography. Air sensitive reactions were conducted in a flame dried glassware under Ar atmosphere. All the solvents used for reactions were distilled before the use.

Iodolactonization of 7 : A mixture of carboxylic acid **7** (1.54 g, 3.2 mmol), iodine (1.31 g, 3.2 mmol) and anhydrous potassium carbonate (2.16 g, 9.6 mmol) in methylene chloride (30 ml) was stirred for 50 min, and saturated sodium thiosulfate (4 ml) was added. The mixture was extracted with ethyl acetate (150 ml), and the organic layer was washed with water (10 ml) and brine (10 ml). The extract was dried over $MgSO_4$ and the solvent was evaporated. The residual oil was purified by colomun chromatography on silica gel. Elution of 30% ethyl acetate in hexane led to a stereoisomeric mixture of iodolactones (**8**α) and (**8**β) (1.87 g) in 89% yield. Although these isomers were more easily separable at the next stage, normal phase (silica gel) hplc eluted by 35% ether in hexane allowed them to be separated. **8**α ; *Oil*; Rf = 0.45 (*30 % EtOAc in hexane*); *ir* (film) *v* 1770 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 7.21 (2H, d, *J* = 8.2 Hz), 6.87 (2H, d, *J* = 8.2 Hz), 4.74 (1H, ddd, *J* = 8.1, 7.2 and 7.0 Hz), 4.44 (1H, d, *J* = 11.5 Hz), 4.38 (1H, d, *J* = 11.5 Hz), 3.79 (3H, s), 3.48 (1H, d, *J* = 9.3 Hz), 3.44 (1H, dd, *J* = 10.3 and 8.1 Hz), 3.39 (1H, d, *J* = 9.3 Hz), 3.15 (1H, dd, *J* = 10.3 and 7.2 Hz), 2.49 (1H, qd, *J* = 7.3 and 7.0 Hz), 1.26 (3H, s), 1.00 (3H, d, *J* = 7.3 Hz); ^{13}C-nmr ($CDCl_3$) δ 178.9, 159.2, 129.4, 129.3, 113.7, 80.4, 73.2, 70.9, 55.2, 48.1, 41.5, 20.5, 8.6, 2.0; LRms *m/z* (rel. intensity, %) 404 (M^+, 59), 277 (21), 137 (base), 121 (90); HRms m/z Calcd for $C_{16}H_{21}O_4I$: 404 0485. Found: 404.0472. **8**β; Oil; Rf = 0.45 (30 % EtOAc in hexane); ir (film) *v* 1770 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 7.19 (2H, d, *J* = 8.7 Hz), 6.86 (2H, d, *J* = 8.7 Hz), 4.44 (1H, d, *J* = 11.6 Hz), 4.33 (1H, d, *J* = 11.6 Hz), 4.00 (1H, ddd, *J* = 9.5, 4.8 and 3.7 Hz), 3.79 (3H, s), 3.53 (1H, dd, J = 11.3 and 3.7 Hz), 3.45 (1H, d, *J* = 9.2 Hz), 3.40 (1H, d, *J* = 9.2 Hz), 3.26 (1H, dd, *J* = 11.3 and 4.8 Hz), 2.05 (1H, dq, *J* = 9.5 and 7.0 Hz), 1.10 (3H, s), 1.08 (3H, d, *J* = 7.0 Hz); ^{13}C-nmr ($CDCl_3$) δ 179.6, 159.3, 129.4, 129.2, 113.8, 81.5, 73.1, 72.9, 55.2, 48.3, 46.1, 19.3, 10.0, 7.3; LRms *m/z* (rel. intensity, %) 404 (M^+, 31), 277 (5), 137 (base), 121 (56); HRms *m/z* Calcd for $C_{16}H_{21}O_4I$: 404.0485. Found: 404.0495.

Deprotection of the PMB group in the mixture of 8α and 8β : To a mixture of **8**α and **8**β (503 mg, 1.24 mmol) in methylene chloride (9 ml) and water (0.5 ml) was added DDQ and the whole mixture stirred for 5 h. The mixture was diluted with methylene chloride (150 ml), washed with water (5 ml X 2) and dried over $MgSO_4$. The solvent was removed and hplc separation (*silica gel, 2.5% ether in methylene chloride as an* eluent) gave less polar isomer (**9**α) (229 mg) in 65% yield, and polar isomer (**9**β) (104 mg) in 29% yield. **9**α ; mp 70.0-71.0°C (recrystallized from benzene:hexane); Rf = 0.32 (40 % EtOAc in hexane); ir (KBr) *v* 3420

and 1740 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 4.82 (1H, ddd, *J* = 8.3, 6.7 and 6.0 Hz), 3.77 (1H, dd, *J* = 11.3 and 4.4 Hz), 3.66 (1H, dd, *J* = 11.3 and 7.1 Hz), 3.48 (1H, dd, *J* = 10.2 and 6.7 Hz), 3.17 (1H, dd, *J* = 10.2 and 8.3 Hz), 2.50 (1H, qd, *J* = 7.3 and 6.0 Hz), 2.21 (1H, dd, *J* = 7.1 and 4.4 Hz), 1.35 (3H, s), 1.06 (3H, d, *J* = 7.3 Hz); ^{13}C-nmr ($CDCl_3$) δ 180.1, 80.6, 63.9, 48.8, 41.7, 19.8, 14.0, 8.5; LRms m/z (rel. intensity, %) 284 (M+, 34), 254 (48), 194 (8), 157 (92), 55 (base); HRms *m/z* Calcd for $C_8H_{13}O_3I$: 283.9910. Found: 283.9909. *Anal.* Calcd for $C_8H_{13}O_3I$: C, 33.82; H, 4.61. Found: C, 34.08; H, 4.71. **9β** ; mp 104.5-105.5°C (recrystallized from benzene:hexane); Rf = 0.32 (40 % EtOAc in hexane); ir (KBr) *ν* 3430 and 1750 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 4.10 (1H, ddd, *J* = 9.4, 5.3 and 3.5 Hz), 3.69 (1H, dd, *J* = 11.0 and 4.7 Hz), 3.61 (1H, dd, *J* = 11.0 and 4.2 Hz), 3.54 (1H, dd, *J* = 11.4 and 3.5 Hz), 3.27 (1H, dd, *J* = 11.4 and 5.3 Hz), 3.10 (1H, br), 2.06 (1H, dq, *J* = 9.4 and 6.9 Hz), 1.16 (3H, d, *J* = 6.9 Hz), 1.05 (3H, s); ^{13}C-nmr ($CDCl_3$) δ 180.7, 82.1, 65.7, 49.6, 45.9, 18.8, 10.0, 7.2; LRms *m/z* (rel. intensity, %) 284 (M^+, 61), 254 (31), 239 (4), 157 (base); HRms *m/z* Calcd for $C_8H_{13}O_3I$: 283.9910. Found 283.9905. *Anal.* Calcd for $C_8H_{13}O_3I$: C, 33.82; H, 4.61. Found: C, 34.03; H, 4.58.

Oxidation of Alcohol (9α and 9β) : To a solution of alcohol (**9α**) (284 mg, 1 mmol) in methylene chloride (8 ml) was added PCC (# J, 1.5 mmol) at room temperature. After stirring for 5 h, the mixture was passed through a florisil column (15 g) to give aldehyde, which was mostly pure and used for the next reaction. Aldehyde obtained from **9α** ; Oil; Rf = 0.50 (40 % EtOAc in hexane); ^{1}H-nmr ($CDCl_3$) δ 9.76 (1H, s), 4.82 (1H, ddd, *J* = 8.5, 6.7 and 5.6 Hz), 3.42 (1H, dd, *J* = 10.4 and 6.7 Hz), 3.17 (1H, dd, *J* = 10.4 and 8.5 Hz), 2.68 (1H, qd, *J* = 7.3 and 5.6 Hz), 1.51 (3H, s), 1.11 (3H, d, *J* = 7.3 Hz). Aldehyde obtained from **9β** ; Oil; Rf = 0.32 (40 % EtOAc in hexane); ^{1}H-nmr ($CDCl_3$) δ 9.56 (1H, d, *J* = 1.5 Hz), 4.07 (1H, ddd, *J* = 9.6, 4.6 and 3.8 Hz), 3.58 (1H, dd, *J* = 11.5 and 3.8 Hz), 3.34 (1H, dd, *J* = 11.5 and 4.6 Hz), 2.32 (1H, dqd, *J* = 9.6 7.1 and 1.5 Hz), 1.57 (3H, s), 1.19 (3H, d, *J* = 7.1 Hz).

Preparation of Iodomethyl Analogues (3α and 3β) : Methylmagnesium bromide (2.2 ml of 0.92 M in THF solution, 2.02 mmol) was dropped to aldehyde, prepared as above from **9α**, in THF (15 ml), for 5 min at 0°C. The reaction was completed instantaneously, and the mixture was quenched with saturated ammmonium chloride solution (10 ml), extracted with ethyl acetate (200 ml). The extract was washed with water (5 ml) and brine (5 ml). The organic extract was dried over $MgSO_4$ and the solvent was evaporated. The crude product was dissolved in acetone (10 ml) and Jones reagent (1.1 ml of 1.85 M solution, 2 mmol)

was added on an ice bath. The mixture was stirred for 15 min, quenched by isopropanol (0.8 ml) and diluted with ethyl acetate (200 ml). The extract was washed with water (10 ml X 2) and brine (10 ml), and dried over $MgSO_4$. Removal of solvent and purification by silica gel chromatography on silica gel gave **3α** (139 mg) in 47% yield in three steps, while the reaction starting from the alternative aldehyde provided **3β** (114 mg) in 39% yield in three steps. **3α** ; mp 110.0-111.0°C (recrystallized from benzene:hexane); Rf = 0.46 (40 % EtOAc in hexane); ir (KBr) ν 1770 and 1700 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 4.75 (1H, ddd, *J* = 7.7, 7.3 and 6.0 Hz), 3.39 (1H, dd, *J* = 10.3 and 7.3 Hz), 3.15 (1H, dd, *J* = 10.3 and 7.7 Hz), 2.59 (1H, qd, *J* = 7.3 and 6.0 Hz), 2.30 (3H, s), 1.52 (3H, s), 1.01 (3H, d, *J* = 7.3 Hz); ^{13}C-nmr ($CDCl_3$) δ 205.9, 175.5, 79.9, 60.0, 43.3, 29.3, 20.6, 9.6, 0.2; LRms *m/z* (rel. intensity, %) 296 (M^+, 5), 254 (base), 239 (3), 169 (59); HRms *m/z* Calcd for $C_9H_{13}O_3I$: 295.9910. Found: 295.9919. *Anal.* Calcd for $C_9H_{13}O_3I$: C, 36.51; H, 4.43. Found: C, 36.64; H, 4.50. **3β** ; mp 88.0-89.0°C (recrystallized from benzene:hexane); Rf = 0.42 (40 % EtOAc in hexane); ir (KBr) ν 1780 and 1690 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 4.02 (1H, ddd, *J* = 9.5, 4.6 and 3.6 Hz), 3.57 (1H, dd, *J* = 11.5 and 3.6 Hz), 3.30 (1H, dd, *J* = 11.5 and 4.6 Hz), 2.22 (3H, s), 2.19 (1H, dq, *J* = 9.5 and 7.0 Hz), 1.52 (3H, s), 1.08 (3H, d, *J* = 7.0 Hz); ^{13}C-nmr ($CDCl_3$) δ 205.2, 175.0, 80.8, 61.1, 48.4, 28.9, 19.2, 11.1, 5.6; LRms *m/z* (rel. intensity, %) 296 (M^+, 1), 254 (base), 239 (5), 169 (7); HRms *m/z* Calcd for $C_9H_{13}O_3I$: 295.9910. Found: 295.9891. *Anal.* Calcd for $C_9H_{13}O_3I$: C, 36.51; H, 4.43. Found: C, 36.80; H, 4.48.

Preparation of 4α and 4β: To a mixture of **3α** (29.6 mg, 0.1 mmol) and tributyltin hydride (38 mg, 0.13 mmol) in benzene (2 ml) was added triethylborane (0.3 ml, 0.1 M in benzene, 0.3 mmol) at room temperature. After 5 min, the mixture was loaded on silica gel chromatography directly and elution by 40% ethyl acetate in hexane gave **4α** (16 mg) in 95% yield. In the same manner, the reduction of **3β** afforded **4β** in 92% yield. **4α** ; mp 53.0-54.0°C (recrystallized from methylene chloride:hexane); Rf = 0.43 (40 % EtOAc in hexane); ir (KBr) ν 1760 and 1700 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 4.72 (1H, dq, *J* = 6.7 and 6.6 Hz), 2.46 (1H, qd, *J* = 7.3 and 6.7 Hz), 2.32 (3H, s), 1.52 (3H, s), 1.32 (3H, d, *J* = 6.6 Hz), 1.02 (3H, d, *J* = 7.3 Hz); ^{13}C-nmr ($CDCl_3$) δ 206.4, 177.0, 77.2, 59.6, 44.2, 29.4, 21.0, 15.7, 10.7; LRms *m/z* (rel. intensity, %) 170 (M^+, 1), 128 (95), 113 (base); HRms *m/z* Calcd for $C_9H_{14}O_3$: 170.0943. Found: 170.0952. **4β** ; mp 70.0-71.0°C (recrystallized from methylene chloride:hexane); Rf = 0.43 (40 % EtOAc in hexane); ir (KBr) ν 1765 and 1690 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 4.32 (1H, dq, *J* = 10.1 and 6.1 Hz), 2.21 (3H, s), 1.95 (1H, dq, *J* = 10.1 and 7.0 Hz), 1.49 (3H, s), 1.40 (3H, d, *J* = 6.1 Hz), 1.04 (3H, d, *J* = 7.0 Hz); ^{13}C-nmr ($CDCl_3$) δ 205.3, 176.5, 80.0, 61.2, 50.4, 29.1, 19.5, 18.6, 10.9; LRms *m/z* (rel. intensity, %) 170 (M^+, 2), 128 (84), 113 (base); HRms *m/z* Calcd for

$C_9H_{14}O_3$: 170.0943. Found 170.0940.

Preparation of 5α and 5β: To a solution of **3α** (15 mg, 0.051 mmol) and a catalytic amount of tetrabutylammonium bromide (a few mg) in ethanol (0.5 ml) was added sodium thiophenolate (1 ml in 0.15M ethanol solution, *ca.* 3 eq.). The mixture was stirred for 2 h at room temperature and then diluted with ether (30 ml), and washed with water (4 ml) and brine (2 ml). The organic layer was dried over $MgSO_4$ and condensed. The crude product was purified by column chromatography on silica gel eluted with 20% ethyl acetate in hexane to give **5α** (13.7 mg) in 97% yield. On the other hand, the reaction of **3β** afforded the corresponding sulfide(**5β**) in 73% yield. **5α** ; mp 121.0-122.0°C (recrystallized from benzene); Rf = 0.43 (40 % EtOAc in hexane); ir (KBr) ν 1770 and 1700 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 7.41 (2H, d, *J* = 7.6 Hz), 7.32 (2H, dd, *J* = 7.6 and 7.3 Hz), 7.25 (1H, t, *J* = 7.3 Hz), 4.60 (1H, ddd, *J* = 7.5, 6.2 and 6.8 Hz), 3.31 (1H, dd, *J* = 13.8 and 6.8 Hz), 3.06 (1H, dd, *J* = 13.8 and 7.5 Hz), 2.60 (1H, qd, *J* = 7.3 and 6.2 Hz), 2.33 (3H, s), 1.51 (3H, s), 1.05 (3H, d, *J* = 7.3 Hz); ^{13}C-nmr ($CDCl_3$) δ 206.1, 176.0, 134.5, 130.4, 129.3, 127.1, 78.4, 59.7, 43.2, 33.8, 29.5, 20.7, 10.5; LRms *m/z* (rel. intensity, %) 278 (M^+, base), 235 (33), 217 (6); HRms *m/z* Calcd for $C_{15}H_{18}O_3S$: 278.0977. Found: 278.0956. *Anal.* Calcd for $C_{15}H_{18}O_3S$: C, 64.72; H, 6.52. Found: C, 64.62; H, 6.76. **5β** ; mp 92.0-93.0°C (recrystallized from benzene); Rf = 0.43 (40 % EtOAc in hexane); ir (KBr) ν 1775 and 1695 cm^{-1}; ^{1}H-nmr ($CDCl_3$) δ 7.40 (2H, d, *J* = 7.6 Hz), 7.31 (2H, dd, *J* = 7.6 and 7.3 Hz), 7.23 (1H, t, *J* = 7.3 Hz), 4.43 (1H, ddd, *J* = 9.8, 5.4 and 4.6 Hz), 3.30 (1H, dd, *J* = 14.3 and 4.6 Hz), 3.21 (1H, dd, *J* = 14.3 and 5.4 Hz), 2.30 (1H, dq, *J* = 9.8 and 7.1 Hz), 2.20 (3H, s), 1.51 (3H, s), 1.07 (3H, d, *J* = 7.1 Hz); ^{13}C-nmr ($CDCl_3$) δ 205.0, 175.6, 135.3, 130.3, 129.2, 127.0, 81.9, 61.0, 47.1, 37.3, 29.0, 19.4, 11.4; LRms *m/z* (rel. intensity, %) 278 (M^+, base), 256 (7), 208 (8), 163 (66); HRms *m/z* Calcd for $C_{15}H_{18}O_3S$: 278.0977. Found: 278.0956. *Anal.* Calcd for $C_{15}H_{18}O_3S$: C, 64.72; H, 6.52. Found: C, 64.61; H, 6.69.

Hydrolysis Test by PLE: To a phosphate buffer solution (2 ml, pH 8.0) of each substrate (2 mg) was added porcine lever esterase (10 units) purchased from Sigma Co. Ltd. The mixture was stirred at room temperature, and the progress was monitored by tlc and hplc.

Biological Activity: Cytotoxicities against L-1210, P-388 and KB cells were examined *in vitro*. The experiments were carried out by the same method reported in the literature.[12, 13]

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