Tetrahedron: Asymmetry Vol. 4, No. 1, pp. 101-108, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

Preparation of 2(R) and 2(S) Methyl-2-Methylglycerates

Juan B. Rodríguez[a], Sanford P. Markey[a] and Herman Zifferb

[a] Laboratory of Clinical Science, NIMH, National Institutes of Health , Bethesda, Md, 20892.

[b] Laboratory of Chemical Physics, NIDDK, National Institutes of Health , Bethesda, Md, 20892.

(*Received in UK* 18 *November* 1992)

Abstract: Optically pure samples of both enantiomers of methyl 2,3-dihydroxy-2-methylpropanoate were prepared by a chromatographic separation of their (-)-menthyl esters followed by hydrolysis and methylation. Their absolute stereochemistries were established by conversion into their 3-*O*-acetyl derivatives, compounds of established configuration. NMR and CG-MS methods of determining the optical purities of the diols are described.

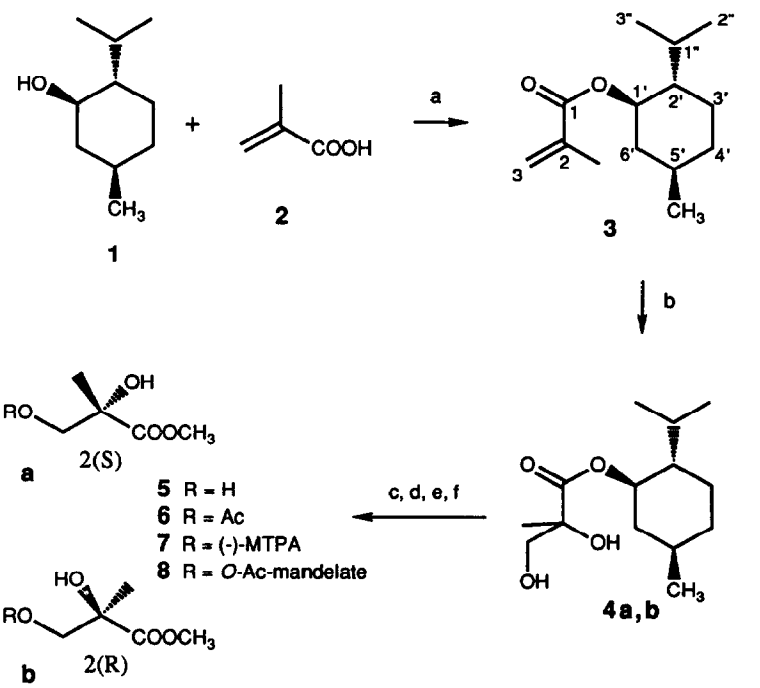
Introduction

Ionizing radiation, mutagenic chemicals and other events cause structural changes in the bases of DNA[1], which if permitted to accumulate would result in serious genetic damage. Molecular biologists have identified a number of DNA polymerases that are responsible for proofreading each DNA strand and excising altered nucleic acids prior to copying the DNA strand[2]. Despite the presence of these repair enzymes, some modified nucleic acids survive and their presence is believed to cause disease. For these reasons, there is interest in identifying and quantifying these compounds in selected samples of DNA. The pyrimidine bases are more readily damaged than purine bases[3]. The thymine bases in DNA are particularly susceptible to radiation damage and undergo oxidative conversion to 5,6-dihydroxy-5,6-dihydrothymine as one major DNA damage process[4]. The relative stereochemistry of this thymine glycol was found to be *cis*[5].

The recent report by Toda *et al.*[6] that irradiation of a prochiral precursor in a chiral crystalline environment yields a chiral product suggests that the glycol product obtained from the reaction of thymine on helical DNA with HO· free radicals would yield an optically active glycol. Quantitative measurements of the amount of methyl 2-methylglycerate **5a,b** have been used as a means for determining radiation damage to DNA[7]. Thymine glycol residues in DNA are easily transformed into **5** by treatment with alkaline sodium borohydride followed by methanolic HCl[7]. In order to determine whether the reaction of thymine in DNA with HO· radicals also produces optically active products, samples of both enantiomers of methyl 2,3-dihydroxy-2-methylpropanoate were required. While compounds **5a** and **5b** have not been prepared in scalemic form , the 3-*O*-acetyl derivative (**6b**) has been synthesized from partially resolved atrolactic acid (2-hydroxy-2-phenylpropionic acid)[8]. We describe here the preparation of both

enantiomers of methyl 2,3-dihydroxy-2-methylpropanoate, their conversion into compounds of established absolute stereochemistry and a CG-MS based method of separating and identifying diasteromeric derivatives.

Scheme 1



a- *p*-TsOH, benzene, reflux, 80%; b- OsO_4 (cat), *N*-methylmorpholine *N*-oxide, H_2O-acetone-*t*-BuOH, 92%; c- column chromatography; d- K_2CO_3, MeOH-H_2O; e- MeOH (anh.)/HCl (gas), 100%; f- for **6** Ac_2O, pyridine, 0°C, 89%; f- for **7** (-)-MTPA-Cl, pyridine, r.t., 95%; f- for **8** (R)-*O*-Acetylmandelic acid, DDC, DMAP, Cl_2CH_2, 0°C, 76%.

RESULTS AND DISCUSSION

There are two general procedures for the preparation of an optically active compound, a) preparation of a diastereomeric mixture and resolution by conventional methods or b) enantioselective synthesis using a chiral auxiliary or chiral reagent in order to induce asymmetry in a prochiral substrate.

A successful asymmetric preparation of 2,3-dihydroxy-2-methylpropanoate derivatives was effected by dihydroxylating the (-)-menthyl ester of methacrylic acid. (-)-Menthol (**1**) was esterified with methacrylic acid (**2**) in benzene in the presence of *p*-toluenesulfonic acid[9] to yield **3** which was dihydroxylated with osmium tetroxide in the presence of *N*-methylmorpholine-*N*-oxide[10] to afford compounds **4a,b** as a

diastereomeric mixture (30% diastereomeric excess of **4a**, analyzed by ^{1}H-NMR spectra of **4a** and **4b**). The most important virtue of the method was that the mixture could be separated by column chromatography. Each diastereomer was saponified with potassium carbonate in water-methanol[11]. The free acids were not isolated but were treated with HCl (g) and methanol[12] to yield the respective methyl esters **5a** and **5b**, each in 100% enantiomeric excess. The absolute configuration of these compounds was determined by comparison of the optical rotation of their 3-*O*-acetyl derivatives with literature data[8]. Compounds **5a** and **5b** were regioselectively monoacetylated by treatment with one equivalent of acetic anhydride in pyridine at 0°C yielding pure acetates **6a** and **6b**. Compound **6a** was assigned the 2(S) configuration while compound **6b** was assigned the 2(R). The enantiomeric excess was determined for **5a** and **5b** by the Mosher's ester derivatives (compounds **7a** and **7b**). These esters were prepared by treatment of diols **5a** and **5b** with (-)-α-methoxy-α-trifluoromethylphenylacetyl chloride[13] in pyridine (see Scheme 1). ^{1}H-NMR analysis of **7a** and **7b** showed that both compounds diols were obtained with 100 % optical purity since there was a 0.09 ppm difference in the chemical shift of the methyl ester group.

Compounds **7a** and **7b** were easily separated by gas chromatography. and detected by mass spectrometry. This is the basis of the method we propose to use to determine the absolute configuration of thymine glycol produced during oxidative DNA damage. The thymine glycol residues are transformed into methyl 2-methylglycerate (**5**), which gives the corresponding Mosher esters. GC-MS analysis and comparison with compounds **7a** and **7b** would give the absolute stereochemistry of **5**. An alternative procedure for assigning the stereochemistry of thymine glycol would involve use of HPLC analysis of *O*-acetylmandelate derivatives **8a** and **8b**, prepared from **5a,b**, **5a** and **5b** [14].

The recent appearance of the Sharpless reagents AD Mix α and AD Mix β[15,16] that have been employed for converting an olefin into an optically active dihydroxy derivative of a predictable configuration prompted us to use the reagents to oxidize methyl methacrylate and *tert*-butyldimethylsilyl methacrylate[17]. In each case diols with small to vanishing enantiomeric excesses were obtained. These results can be understood considering the rule based on the relative sizes of the substituents at the double bond described by Sharpless *et al.*[16] for predicting the configuration of the products. Differences in the relatives sizes of a methyl and carboxymethyl groups may not be large enough for ligands to influence complex formation and reaction. In the case of the silyl methacrylate, hydrolysis of the silyl ester before perhydroxylation was observed, probably due to presence of Fe^{3+} in the reaction medium.

EXPERIMENTAL

NMR spectra were recorded with a Varian Gemini 300 at 300 MHz. Optical rotations were performed in a Perkin-Elmer Model 241 MC polarimeter. HPLC studies employed a Hewlett-Packard Model 1090

with a diode array detector using an Altex Ultrasphere ODS-2 5 mm column (250 x 10 mm). GC-FTIR spectra were obtained using a Hewlett-Packard Model 5965A instrument with a narrow band detector (4000-750 cm^{-1}) and a 59970 IRD ChemStation interfaced with a Hewlett-Packard Model 5890 gas chromatograph having a 25 m x 0.32 mm HP-5 (bonded 5% diphenylsiloxane : 95% dimethylsiloxane) fused silica capillary column. CG-MS were measured with a Finnigan Model 800 ion trap mass detector interfaced with a Varian 3400 gas chromatograph equipped with a similar column as used for GC-FTIR using a program from 100°C to 280°C at 10°C/min to generate the total response chromatogram. A Finnigan 4600 mass spectrometer was used in chemical ionization mode with ammonia as a reagent gas. Elemental analysis were performed by Galbraith Laboratory, Inc.

Menthyl 2-methyl propenoate **3**

To a solution of methacrylic acid **2** (5 mmol) and (-)-menthol **1** (5 mmol) in 100 ml of benzene, *p*-toluenesulfonic acid (0.1 mmol) was added and the mixture was refluxed for two days employing a Dean-Stark trap. The mixture was cooled to room temperature and was washed with a saturated solution of $NaHCO_3$ (3 x 40 ml) and water (2 x 30 ml). The organic layer was dried ($MgSO_4$) and the solvent was evaporated. The residue was purified by flash chromatography eluting with hexane-ethyl acetate (98:2) affording pure compound **3** as a colorless oil in 80% yield.
IR (cm^{-1}) = 2964, 2880, 1730, 1638, 1460, 1381, 1297, 1188, 1092, 980, 810. ^{1}H-NMR ($CDCl_3$) δ = 0.76 (d, J = 7.0 Hz, 3H, Me at C-5'); 0.90 (d, J = 7.1 Hz, 3H, H-2"); 0.91 (d, J = 6.5 Hz, 3H, H-3"); 1.94 (t, J = 1.5 Hz, 3H, Me at C-2); 4.73 (dt, J_1 = 4.4 Hz, J_2 = 10.8 Hz, H-1'); 5.52 (t, J = 1.5 Hz, 1H, H-3A); 6.08 (s,1H, H-3B). ^{13}C-NMR ($CDCl_3$) δ = 16.57 (C-3"); 18.48 (Me at C-2); 20.83 (C-C2"); 22.11 (Me at C-5'); 23.68 (C-3'); 26.52 (C-1"); 31.45 (C-5'); 34.37 (C-4'); 40.91 (C-6'); 47.19 (C-2'); 74.44 (C-1'); 124.76 (C-3); 136.78 (C-2); 166.83 (C-1). CIMS (m/z, %) = 242 ($\{M+NH_3+1\}^+$, 45); 225 (30); 156 (30); 138 (100). *Anal.* Calcd for $C_{14}H_{24}O_2$: C 74.95; H 10.78. Found: C 75.03; H 10.77.

Menthyl 2(S),3-dihydroxy-2-methylpropanoate **4a** and *menthyl 2(R),3-dihydroxy-2-methylpropanoate* **4b**.

To a mixture of *N*-methylmorpholine-*N*-oxide.2 H_2O (490 mg) and osmium tetroxide (30 mg) in 28 ml of water-acetone (5:2) compound **3** (896 mg) in 3.2 ml of *tert*-butanol was added. The reaction was complete after stirring overnight at room temperature under argon atmosphere. A slurry of 1 g of sodium hydrosulfite, 1 g of Magnesol and 40 ml of water was added and the Magnesol was filtered. The filtrate was neutralized to pH 7 with 1N H_2SO_4, the acetone was evaporated under vacuum and the pH was further adjusted to pH 2. The solution was saturated with sodium chloride and extracted with ethyl acetate (3 x 30 ml). The collected organic layers were dried ($MgSO_4$) and the solvent was evaporated to afford 949 mg of diol **4** as a diasteromeric mixture (30% diasteromeric excess of **4a**). The mixture was separated by column chromatography using hexane-ethyl acetate (85:15) as eluent to yield 250 mg of pure **4a**, 140 mg of **4b** and unresolved mixture as colorless oils.

Data of **4a** 2(S) (fraction # 1, < R_f): $[\alpha]_D$ = - 59.1 (c 2.0, ethanol). IR (cm^{-1}) = 3567, 3071, 2962, 2855, 1765, 1453, 1373, 1188, 1125, 1030. ^{1}H-NMR ($CDCl_3$) δ = 0.77 (d, *J* = 6.8 Hz, 3H, Me at C-5'); 0.907 (d, *J* = 7.0 Hz, 3H, H-2"); 0.913 (d, *J* = 6.4 Hz, 3 H, H-3"); 1.35 (Me at C-2); 3.57 (d, *J* = 11.2Hz, 1H, H-3A); 3.78 (d, *J* = 11.2 Hz, 1H, H-3B); 4.77 (dt, J_1 = 4.4 Hz, J_2 = 11.0 Hz, 1H, H-1'). ^{13}C-NMR ($CDCl_3$) δ = 16.07 (C-3"); 20.85 (C-2"); 21.99 (Me at C-2); 23.25 (C-3'); 26.29 (C-1"); 31.67 (C-5'); 34.18 (C-4'); 40.56 (C-6'); 46.97 (C-2'); 68.36 (C-3); 75.34 (C-2); 76.65 (C-1'); 175.15 (C-1). CIMS (m/z, %) = 276 ({M+NH_3+1}$^+$, 100). *Anal.* Calcd for $C_{14}H_{26}O_4$: C 65.09; H 10.14. Found: C 65.01; H 10.12.

Data of **4b** 2(R) (fraction # 2, > R_f): $[\alpha]_D$ = - 61.5 (c 2.0, ethanol). IR (cm^{-1}) = 3569, 3071, 2962, 2855, 1765, 1453, 1373, 1184, 1126, 1030. ^{1}H-NMR ($CDCl_3$) δ = 0.76 (d, *J* = 6.8 Hz, 3H, Me at C-5'); 0.91 (t, *J* = 6.2 Hz, 6H, H-2" & H-3"); 1.34 (s, 3H, Me at C-2); 3.58 (d, *J* = 11.2 Hz, 1H, H-3A); 3.78 (d, *J* = 11.2 Hz, 1H, H-3B); 4.77 (dt, J_1 = 4.4 Hz, J_2 =11.0 Hz, 1H, H-1'). ^{13}C-NMR ($CDCl_3$) δ = 15.88 (C-3"); 20.76 (C-2"); 21.94 (Me at C-2); 23.10 (C-3'); 25.95 (C-1"); 31.36 (C-5'); 34.12 (C-4'); 40.58 (C-6'); 46.91 (C-2'); 68.16 (135.38); 75.49 (C-2); 76.40 (C-1'); 175.12 (C-1). CIMS (m/z, %) = 276 ({M+NH_3+1}$^+$, 100).

Methyl-2(S),3-dihydroxy-2-methyl propanoate **5a** and *methyl-2(S),3-dihydroxy-2-methyl propanoate* **5b**.

To 200 mg of compound **4a** (0.78 mmol) in methanol (10 ml) 465 mg of potassium carbonate (anhydrous powder) were added while stirring, followed by water (3 ml) to obtain complete solution. After two days at room temperature, the reaction mixture was washed with CH_2Cl_2 (3 x 25 ml). The aqueous layer was evaporated to dryness under reduced pressure. The residue was triturated with anhydrous methanol (20 ml) and and the solution was filtrated. Then HCl (g) was bubbled for 10 minutes. The reaction mixture was stirred for 60 minutes and then the solvent was evaporated. The residue was triturated with CH_2Cl_2 (30 ml) and the solution was filtered. Evaporation of the solvent yielded 103 mg of pure diol **5a** as a yellow pale oil. Compound **5b** was obtained in a similar yield from 80 mg of **4b**.

$[\alpha]_D$ = +2.8 (c 3.0, ethanol) for **5a** ; $[\alpha]_D$ = -2.9 (c 3.0, ethanol) for **5b**. IR (cm^{-1}) = 3625, 3550, 2961, 2884, 1752, 1451, 1385, 1218, 1058, 983.^{1}H-NMR ($CDCl_3$) δ = 1.36 (s, 3H, Me at C-2); 3.58 (d, *J* = 11.2 Hz, 1H, H-3A); 3.81 (d, *J* = 11.2 Hz, 1H, H-3B); 3.82 ($COOCH_3$). ^{13}C-NMR ($CDCl_3$) δ = 21.78 (Me at C-2); 52.87 ($COOCH_3$); 68.38 (C-3); 75.79 (C-2); 175.78 (C-1). CIMS (m/z, %) = 152 ({M+NH_3+1}$^+$, 100); 138 (18).

Methyl-3-O-acetyl-2(S),3-dihydroxy-2-methylpropanoate **6a** and *Methyl-3-O-acetyl-2(S),3-dihydroxy-2-methylpropanoate* **6b**.

A solution of 46 mg of compound **5a** in 0.5 ml of pyridine was treated with acetic anhydride (45 ml) and the reaction mixture was stirred overnight at 0°C. The mixture was allowed to warm to room temperature, CH_2Cl_2 (3 ml) and water (3 ml) were added and the mixture stirred at room temperature for

an additional hour. The organic layer was washed with 1N HCl (3 x 3 ml), water (2 x 3 ml), dried ($MgSO_4$) and the solvent evaporated. The residue was purified by column chromatography employing hexane-ethyl acetate as eluent affording 54 mg (89% yield) of pure acetate **6a** as a colorless oil .

The same procedure was followed in obtaining the respective enantiomeric derivative from 30 mg of compound **5b** affording the corresponding acetate **6b** in a similar yield.

$[\alpha]_D$ = +9.3 (c 6.0, ethanol) for **6a**; $[\alpha]_D$ = -9.4 (c 3.0, ethanol) for **6b**; lit[4] $[\alpha]_D$ = -7.33 (c 6.09, ethanol) for **6b**[18]. IR (cm^{-1}) = 3574, 2962, 1765, 1453, 1376, 1226, 1049, 984. ^{1}H-NMR ($CDCl_3$) δ = 1.43 (s, 3H, Me at C-2); 2.07 (s, 3H, CH_3CO); 3.81 (s, 3H, OCH_3); 4.14 (d, J = 11.2 Hz, H-3A); 4.27 (d, J = 11.2 Hz, H-3B). ^{13}C-NMR ($CDCl_3$) δ = 20.69 (CH_3CO); 22.18 (Me at C-2); 53.04 (OCH_3); 69.15 (C-3); 73.57 (C-2); 170.44 (C-1); 174.70 ($COCH_3$). CIMS (m/z, %) = 194 ({M+NH_3+1}$^+$, 100). *Anal.* for **6a** Calcd for $C_7H_{12}O_5$: C 47.73; H 6.87. Found: C 47.83; H 6.95.

Methyl-3-O-(α-methoxy-α-trifluoromethylphenylacetyl)-2(S),3-dihydroxy-2-methylpropanoate **7a** and *methyl-3-O-(α-methoxy-α-trifluoromethylphenylacetyl)-2(R),3-dihydroxy-2-methylpropanoate* **7b**.

Enantiomeric pure diols **4a** and **4b** (15 mg each), dissolved in 0.5 ml of anhydrous pyridine were treated, in separate experiments, with (-)-α-methoxy-α-trifluoromethylphenylacetyl chloride (35 mg) and the mixtures were stirred overnight at room temperature. The reaction mixtures were dissolved in CH_2Cl_2 (10 ml) and washed with 1N HCl (3 x 10 ml), a saturated solution of $NaHCO_3$ (2 x 10 ml), and water (2 x 10 ml). The organic layers were dried ($MgSO_4$) and the solvent evaporated to afford **7a** and **7b** in 95 % yield (oils). CG-MS analysis of **7a** and **7b** showed that the 2(S) derivative **7a** had a shorter retention time than the 2(R) derivative **7b**.

Data of **7a** 2(S): $[\alpha]_D$ = -35.0 (c 2.0, ethanol). IR (cm^{-1}) = 3567, 3071, 2962, 2855, 1765, 1453, 1373, 1103, 1125, 1030. ^{1}H-NMR ($CDCl_3$) δ = 1.44 (s, 3H, Me at C-2); 3.53 (m, 3H, OCH_3); 3.66 (s,3H, $COOCH_3$); 4.37 (d, J = 11.5 Hz, 1H, H-3A); 4.45 (d, J = 4.5 Hz, 1H, H-3B); 7.35-7.55 (m, 5H, aromatic protons). ^{13}C-NMR ($CDCl_3$) δ = 22.37 (Me at C-2); 53.17 ($COOCH_3$); 55.43 (OCH_3); 70.70 (C-3); 73.34 (C-2); 127.28 (C-6'); 128.35 (C-4'); 129.59 (C-5'); 130.39 (C-3'); 165.84 (C-1'); 174.26 (C-1). EIMS (ion trap) (m/z, %) = 333 ({M^+-17}, 10); 259 (25); 189 (100); 105 (35).

Data of **7b** 2(R): $[\alpha]_D$ = -30.9 (c 2.0, ethanol). IR (cm^{-1}) = 3569, 2962, 2856, 1765, 1453, 1372, 1104, 1126, 1030. ^{1}H-NMR ($CDCl_3$) δ = 1.43 (s, 3H, Me at C-3); 3.50 (m, 3H, OCH_3); 3.75 (s, 3H, $COOCH_3$); 4.37 (d, J = 7.2 Hz, 1H, H-3A); 4.42 (d, J = 7.2Hz, 1H, H-3B); 7.35-7.55 (m, 5H, aromatic protons). ^{13}C-NMR ($CDCl_3$) δ = 22.16 (Me at C-2); 55.34 ($COOCH_3$); 55.33 (OCH_3); 70.52 (C-3); 73.29 (C-2); 127.28 (C-6'); 128.35 (C-4'); 129.59 (C-5'); 130.39 (C-3'); 165.84 (C-1'); 174.26 (C-1). EIMS (ion trap) (m/z, %) = 333 ({M^+-17}, 10); 259 (25); 189 (100); 105 (35).

Methyl-3-O-(-(R)-O-acetylmandelyl)-2(S),3-dihydroxy-2-methylpropanoate **8a** and *methyl-3-O-(-(R)-O-acetylmandelyl)-2(R),3-dihydroxy-2-methylpropanoate* **8b**.

To a solution of 388 mg of (R)-*O*-acetylmandelic acid, 270 mg of diol **5a,b** (racemic mixture)[19] and 40 mg of 4-(dimethylamino)pyridine in 10 ml of anhydrous CH_2Cl_2 at 0°C dicyclohexylcarbodimide (420

mg) in CH_2Cl_2 (5 ml) was added dropwise with stirring . A white precipitate of dicyclohexylurea was observed in the reaction medium before the addition was complete. The reaction mixture was stirred for 24 hours at room temperature. The urea was removed by filtration, and the mixture was washed with 1N HCl (3 x 20 ml), 2N K_2CO_3 (3 x 20 ml) and brine (2 x 30 ml). The organic layer was dried ($MgSO_4$) and the solvent evaporated. The residue was purified by column chromatography (silica gel), eluting with hexane-ethyl acetate (4:1) to yield **8a,b** as diastereomeric mixture in 76 % yield. The mixture was finally separated by HPLC employing MeOH-H_2O (2:3) as eluent at a rate of 3.00 ml/min. In order to identify both diastereomers, pure **8a** and **8b** were prepared from **5a** and **5b** respectively in a similar yield. The peak with the slower retention time corresponded to the 2(S) derivative (compound **8a**). The peak with faster retention time was assigned the 2(R) configuration (compound **8b**).

Data of **8a**: IR (cm^{-1}) = 3571, 2693, 1766, 1456, 1227, 1171, 1055. ^{1}H-NMR ($CDCl_3$) δ = 1.36 (s, 3H, Me at C-2), 2.19 (s, 3H, C<u>H</u>3CO-); 3.53 (s, 3H, OC<u>H</u>3); 4.13 (d, *J* = 11 Hz, 1H, H-3A); 4.33 (d, *J* = 11 Hz, 1H, H-3B); 5.90 (s, 1H, H-2'); 7.35-7.45 (m, 5H, aromatic protons). ^{13}C-NMR ($CDCl_3$) δ = 20.71 (OC(O)<u>C</u>H3); 21.88 (Me at C-2); 52.93 (O<u>C</u>H3); 70.08 (C-3); 73.58 (C-2); 74.42 (C-2'); 127.46 (C-5'); 128.72 (C-4'); 129.20 (C-6'); 133.43 (C-3'); 168.09 (C-1'); 170.17 (CH3<u>C</u>O-); 174.37 (C-1). EIMS (ion trap) (m/z, %) = 293 ({M^+-17}, 53); 223 (100); 176 (45); 159 (62); 107 (30).

Data of **8b**:IR (cm^{-1}) = 3576, 2693, 1766, 1456, 1227, 1171, 1055. ^{1}H-NMR ($CDCl_3$) δ = 1.35 (s, 3H, Me at C-2); 2.20 (s, 3H, C<u>H</u>3CO-); 3.75 (s, 3H, OC<u>H</u>3); 4.24 (d, *J* = 11.2 Hz, 1H, H-3A); 4.29 (d, *J* = 11.2 Hz, 1H , H-3B); 5.88 (s, 1H, H-2'); 7.38-7.45 (m, 5H, aromatic protons). ^{13}C-NMR ($CDCl_3$) δ = 20.71 (OC(O)<u>C</u>H3); 22.01 (Me at C-2); 53.16 (O<u>C</u>H3); 69.75 (C-3); 73.49 (C-2); 74.35 (C-2'); 127.51 (C-5'); 128.72 (C-4'); 129.28 (C-6'); 168.09 (C-1'); 170.31 (CH3<u>C</u>O-); 174.45 (C-1). EIMS (ion trap) (m/z, %) = 293 ({M^+-17}, 53); 223 (100); 176 (45); 159 (62); 107 (30).

Acknowledgments : Thanks are owed to Dr. H.M. Garraffo and Dr. T. Spande for the use of HPLC, CG-MS and CG-FTIR instruments. We are also indebted to Dr. T. A. Santa-Coloma for very helpful suggestions.

REFERENCES AND NOTES

1- R. Cathcart, E. Schwiers, R. L. Saul & B. N. Ames *Proc. Natl. Acad. Sci. USA,* **1984**, *81*, 5633.

2- J. Darnell, H. Lodish, D. & Baltimore, *Molecular Cell Biology*; Scientific American Books: New York. 1986. pp. 517-568.

3- M. Sharma, H.C. Box & D.J. Kelman, *Chem Biol. Interactions*, **1990**, *74*, 107.

4- a-H. Ide, Y. W. Kow & S.S. Wallace, *Nucleic Acid Res.*, **1985**,*13*, 8035.
b-J.M. Clark & G.P. Beardsley, *Nucleic Acid Res.*, **1986**,*14*, 737.
c-H. Ide, R.J. Melamede & S.S. Wallace, *Biochemistry*, **1987**, *26*, 964.

5- K. Frenkel, M. S. Goldstein, N. J. Duker & G. W. Teebor, *Biochemistry*, **1981**, *20*, 750.
6- A. Sekine, K. Hori, Y. Ohashi, M. Yagi & F. Toda, *J. Am. Chem. Soc.*, **1989**, *111*, 697
7- K. A. Schellenberg & J. Shaeffer, *Biochemistry*, **1986**, *25*, 1479.
8- F. L. Shore & G. U. Yuen, *J. Org. Chem.*, **1972**, *37*, 3703.
9- J. F. Carson, *Synthesis*, **1979**, 24.
10- V. van Rheenen, R.C. Kelly & D.Y. Cha, *Tetrahedron Lett.*, **1976**, *23*, 1973.
11- W.F. Huffman, R.F. Hall, J.A. Grant & K.G. Holden, *J. Med. Chem.*, **1978**, *21*, 413.
12- K.P. Lippke, W.G. Schunack, W. Wenning & W. E. Müller, *J. Med. Chem.*, **1983**, *26*, 499.
13- J.A. Dale, D. L. Dull & H. S. Mosher, *J. Org. Chem.*, **1969**, *34*, 2543.
14- J. K. Whitesell & D. Reynolds, *J. Org. Chem.*, **1983**, *48*, 3548.
15- K. B. Sharpless, W. Amberg, M. Beller, H. Chen, J. Hartung, Y. Kawanami, D. Lübben, E. Manoury, Y. Ogino, T. Shibata & T. Ukita, *J. Org. Chem.*, **1991**, *56*, 4585.
16- K.B. Sharpless, W. Amberg, Y.L. Benani, G.A. Crispino, J. Hartung, K.S. Jeong, H.L. Kuong, K. Morikawa, Z.M. Wang, D. Xu & X.L. Zhang, *J. Org. Chem.*, **1992**, *57*, 2768.
17- Prepared by the method described by E.J. Corey & A. Venkateswarlu, *J. Am. Chem. Soc.*, **1972**, *94*, 6190. ^{1}H-NMR ($CDCl_3$) δ = 0.30 (s, 6H, Si($C\underline{H}_3$)$_2$); 0.96 (s, 9H, *t*-BuSi); 1.93 (d, J = 1.6 Hz, 3H, Me at C-2); 5.58 (m, 1H, H-3A); 6.10 (d, J < 1 Hz, 1H, H-3B). ^{13}C-NMR ($CDCl_3$) δ = -4.76 (Si($\underline{C}H_3$)$_2$); 18.39 (Me at C-2); 25.63 (*t*-BuSi); 125.82 (C-3); 137.62 (C-2); 167.38 (C-1).
18- The authors reported this value for 77.1% optical purity and they predicted a value of -9.52 for an optically pure sample. They also presented $[\alpha]_D$ = -9.18 (c 5.07, ethanol) for this compound, prepared by other method assuming that its optical purity was 97.5 % and they also predicted $[\alpha]_D$ = -9.42 for a pure sample.
19- Prepared by the method described by D. Swern, J.T. Scanlon & G.B. Dickel, *Org. Synt. Coll. Vol IV*, **1963**, 317.