Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 11

NEW STEREOSPECIFIC LABELING OF MEVALONOLACTONE AT POSITION C-5 WITH DEUTERIUM AND TRITIUM

Lolita O. Zamir* and Cong-Danh Nguyen
Université du Québec, Institut Armand-Frappier
531, boul. des Prairies, Ville de Laval, Québec, H7V 1B7

SUMMARY

The synthesis of (3RS)(5R)[5-^{2}H]-,(3RS)(5S)[5-^{2}H]-and (3RS)(5S)[5-^{3}H]-mevalonolactones are described.

Key words: Mevalonolactone. Stereospecific labeling with deuterium and tritium.

INTRODUCTION

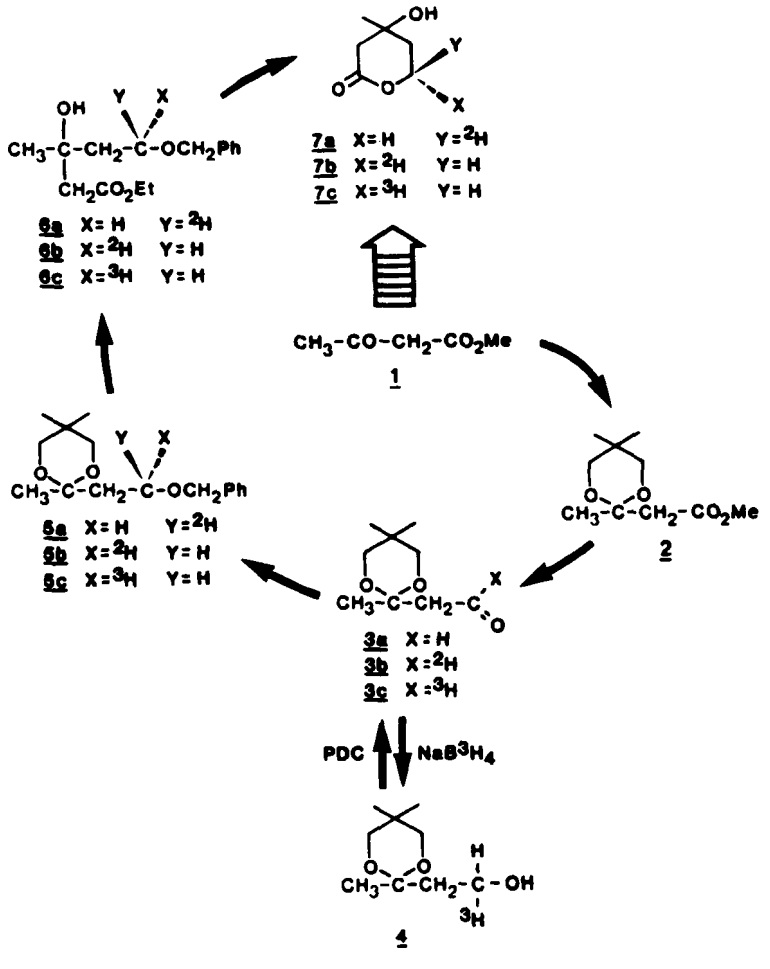
Mevalonolactone is the essential and universal precursor to terpenoid compounds, giving rise to the "isoprene" unit by a decarboxylation-dehydration reaction. During our investigation on the detailed mechanism of trichothecene biosynthesis (1,2,3), enantiospecific labeling of mevalonate at C-5 was required. An early elegant synthesis had been described in the literature (4) and later applied (5). The main drawback of this method is that it is based on an intermediate (3-methyl-but-3-en-1-al) which is very volatile, unstable to heat and base and can easily isomerize to the α-β unsaturated aldehyde. The overall yield reported is 3.2%. In a preliminary communication (6) we have reported a new route to (3RS)(5R)[5-^{2}H]- and (3RS)(5S)[5-^{2}H]-mevalonolactones using very stable intermediates, simple routes and higher yields (35%). We have successfully extended these syntheses to the respective tritiated mevalonolactones. The synthesis of mevalonolactone stereospecifically labeled at carbon 5 combines chemical reactions with an

* Author to whom correspondence should be addressed.

0362–4803/88/111189–08$05.00

Received October 13, 1987

enzymatic reduction step (7). An effective auto-recycling system was used for the enzymatic step eliminating the need to prepare NAD²H or NAD³H. The present publication represents the full paper describing in detail these results. The different steps involved in the synthesis are illustrated in scheme 1:



SCHEME 1

RESULTS AND DISCUSSION

Commercially available methyl acetoacetate (1) was first acetalized to 2,5,5-trimethyl-2-carbomethoxylmethyl-1,3-dioxane (2) by refluxing in dichloromethane with trimethylchlorosilane (TMSCl) and 2,2-dimethyl-1,3-propanediol. This quantitative

acetalization is analogous to that described for carbonyl compounds by Chan (8). Derivative 2 was then successively reduced with $LiAlH_4$ (or $LiAlD_4$) and oxidized with pyridinium dichromate (PDC) in CH_2Cl_2 to give 2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-acetaldehyde (3a) (or [1-^{2}H]-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-acetaldehyde (3b)). The overall yield was 63%. [1-^{3}H]-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-acetaldehyde (3c) was easily prepared from intermediate 3a by reduction with NaB^3H_4 to [1-^{3}H]-2-(2,5,5,-trimethyl-1,3-dioxane-2-yl)-ethanol (4), followed by oxidation of the latter with PDC. We found that the key acetaldehyde derivatives 3a, 3b and 3c were perfectly suitable for subsequent enzymatic reduction with horse liver alcohol dehydrogenase (HLAD) to the corresponding chiral alcohols. Thus, compound 3a was converted to (1R)[1-^{2}H]-1-benzyloxy-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-ethane (5a) by treatment with HLAD, nicotinamide adenine dinucleotide (NAD+), ethyl alcohol-d_6 in 0.05M KH_2PO_4 buffer, pH 8.8, followed by the usual benzylation of the resulting alcohol (NaH, benzyl chloride, N,N-dimethylformamide). The overall yield was 65%. The same reaction sequence with non-deuterated ethyl alcohol afforded (1S)[1-^{2}H]-1-benzyloxy-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-ethane (5b) and (1S)[1-^{3}H]-1-benzyloxy-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-ethane (5c) from 3b and 3c respectively. Deprotection of compounds 5a-c with methanolic hydrogen chloride, followed by condensation of the resulting ketone with ethyl acetate, lithium di-isopropylamide (LDA) in tetrahydrofuran (THF) at -78°C gave respectively ethyl (3RS)(5R)[5-^{2}H]-3-hydroxy-3-methyl-5-benzyloxy-pentanoate (6a), ethyl (3RS)(5S)[5-^{2}H]-3-hydroxy-3-methyl-5-benzyloxy-pentanoate (6b) and ethyl (3RS)(5S)-[5-^{3}H]-3-hydroxy -3-methyl -5-benzyloxy-pentanoate (6c). The yield was 93%. Finally, upon debenzylation by hydrogenolysis on a palladium catalyst in methanolic hydrogen chloride at pH~3, compounds 6a-c cyclized to give the corresponding lactones (3RS)(5R)[5-^{2}H]-mevalonolactone (7a), (3RS)(5S)[5-^{2}H]-mevalonolactone (7b), and (3RS)(5S)[5-^{3}H]-mevalonolactone (7c). The yield was 86%. The structures of all the intermediates were assigned by their infrared spectra (IR), proton nuclear magnetic resonances (^{1}H-NMR) and mass spectrometry analyses (see experimental section). On the other hand unlabeled mevalonolactone synthesized with unlabeled reagents by the previously described procedure was identical in all respect to an authentic sample of mevalonolactone.

EXPERIMENTAL

Instrumentation

^{1}H-NMR spectra were recorded at 60MHz in deuteriochloroform containing tetramethyl silane as internal reference. IR spectra were obtained in chloroform solution on a Perkin Elmer 683 spectrophotometer. Mass spectra (MS) were taken on a HP5980A mass spectrometer at 70ev.

Materials

Flash chromotography was performed using silica gel (200-400 mesh) purchased from B.D.H. Chemicals. THF was freshly distilled over lithium aluminium hydride. DMF and di-isopropylamine were distilled on calcium hydride. Horse liver alcohol dehydrogenase and β-nicotinamide adenine dinucleotide were purchased from Sigma.

2,5,5-Trimethyl-2-carbomethoxymethyl-1,3-dioxane (2)

Under argon atmosphere, 40g (34.44 mmol) of methylacetoacetate and 7.89g (75.87 mmol) of 2,2-dimethylpropane-1,3-diol were mixed with 160 ml of dry methylene chloride. Next, 151.52 mmol of TMSCl were added dropwise. The obtained mixture was then refluxed overnight. After neutralization of the reaction media with 5% aqueous $NaHCO_3$, the organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. Flash chromatography with ethyl acetate-hexane (1:4 v/v) gave pure acetal 2 with a yield of 98%.

^{1}H-NMR ($CDCl_3$) δ (ppm): 1.00 (s, 6H, gem CH_3); 1.60 (s, 3H, $\underline{CH_3}$-CO_2-); 2.80 (s, 2H, $\underline{CH_2}$-CO_2-); 3.60 (s,4H, $O\underline{CH_2}$-C-$\underline{CH_2}O$); 3.65 (s, 3H, $CO_2\underline{CH_3}$). IR ($CHCl_3$) ν(cm^{-1}): 1735, 2960. MS: m/e 203 $[M + 1]^+$, 129 $[M-CH_2CO_2Me]^+$.

2-(2,5,5-Trimethyl-1,3-dioxane-2-yl)-acetaldehyde (3a)

Under argon, 3g (14.85 mmol) of acetal 2 in 5ml of THF were added dropwise at 0°C into a round bottom flask containing 14.85 mmol of $LiALH_4$ in suspension in 50 ml of THF. The mixture was magnetically stirred for 1h, then quenched with 5 ml of water. After filtration and extraction with ether, the organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness to give 2.56g of the corresponding primary alcohol. 1.25g of this primary alcohol without any purification were dissolved in 50 ml of methylene chloride, then added slowly to a

round bottom flask containing 8g of PDC in suspension in 100 ml of the same solvent. After refluxing overnight, the mixture was filtered through a silica gel column. Evaporation of the solvent to dryness was followed by flash chromatography of the crude product with ethyl acetate : hexane (1:4 v/v). The pure aldehyde 3a was obtained in an overall yield of 63%.

^{1}H-NMR ($CDCl_3$) δ (ppm): 0.90 (s, 3H, CH_3); 1.10 (s, 3H, CH_3); 1.50 (s, 3H, CH_3); 2.70 (d, J = 4Hz, 2H, $C\underline{H}_2$-CHO); 3.60 (m, 4H $OC\underline{H}_2$-C-$CH_2\underline{O}$); 9.95 (t, J = 4Hz, 1H, CHO). IR ($C\underline{H}Cl_3$) ν(cm^{-1}): 1720, 2960. MS: m/e 173 $[M + 1]^+$, 129 $[M-CH_2CHO]^+$.

[1-^{2}H]-2-(2,5,5 -Trimethyl-1,3-dioxane-2-yl)-acetaldehyde (3b)

The procedure described for 3a was used but $LiAlH_4$ was replaced with $LiAlD_4$. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.90 (s, 3H, CH_3); 1.10 (s, 3H, CH_3); 1.60 (s, 3H, CH_3); 2.80 (s, 2H, CH_2-CDO); 3.60 (m, 4H $OC\underline{H}_2$-c-$CH_2\underline{O}$); IR ($CHCl_3$) ν(cm^{-1}): 1710, 2960. MS: m/e 174 $[M + 1]^+$.

[1-^{3}H]-2-(2,5,5-Trimethyl-1,3-dioxane-2-yl)-acetaldehyde (3c)

Aldehyde 3a (3.45 mmol) was stored in 10 ml of methanol and cooled at 0°C. NaB^3H_4 (500 mCi) in 2ml of methanol, was added dropwise with a Pasteur pipette. The reduction was complete after addition of an excess (2 to 5 equivalents) of non tritiated $NaBH_4$ (130 mg). The resulting [1-^{3}H]-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-ethanol (4) was extracted with ethyl acetate, washed with brine and dried over $MgSO_4$. After filtration and evaporation to dryness, the crude product (585 mg) was dissolved in 2 ml of CH_2Cl_2, then added dropwise to a round bottom flask containing a suspension of PDC (6.25g) in 50 ml of CH_2Cl_2. The mixture was refluxed overnight, then filtered through a small column of silica gel. After evaporation to dryness below 30°C, aldehyde 3c was purified by flash chromatography with ethyl acetate- hexane (1:4 v/v). The yield was 70%. Compounds 3a-e have the same R_f on TLC plates. The IR spectra of 3a-e was the same.

(1R)[1-^{2}H]-1-Benzyloxy-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)- ethane (5a)

2.43 g (14.04 mmol) of 3a were partially dissolved in 194 ml of 0.05 M KH_2PO_4 buffer, pH 8.8, and 2 ml of fully deuterated ethyl alcohol were added. The addition

of NAD^+ (170 mg) was followed by 146 mg of HLAD (1.5 units/mg). The mixture was magnetically stirred at room temperature overnight. Adjustments of the pH to 8.8 were made at 1h interval during the 4 first hours by addition of diluted aqueous KOH solution. The resulting deuterated primary alcohol was extracted with ether, washed with water and dried over $MgSO_4$. After evaporation to dryness, flash chromatography with ethyl acetate-hexane (1:1 v/v) gave pure deuterated alcohol intermediate (2.08 g). This primary alcohol was then solubilized in 20 ml of DMF and treated with 570 mg of NaH at 0°C during 20 min, followed by addition of benzyl chloride (29 mmol). The resulting mixture was stirred at room temperature overnight. After the usual work-up, and purification by flash chromatography with ethyl acetate-hexane (1:9 v/v), compound 5a was isolated with 65% overall yield. ^{1}H-NMR ($CDCl_3$) δ (ppm): 0.90 (s, 3H, CH_3); 1.00 (s, 3H, CH_3); 1.20 (s, 3H, CH_3); 2.10 (d,J = 8Hz, 2H, $C\underline{H}_2$-CDH); 3.50 (s, 4H $OC\underline{H}_2$-C-$\underline{CH}_2O$); 3.60 (t, J = 8Hz, 1H, CH_2-C$\underline{D}$HO); 4.50 (s, 2H, $\underline{CH}_2$Ph); 7.30 (s, 5H, Ph). IR ($CHCl_3$) ν(cm^{-1}): 700, 2140, 2960. MS: m/e 266 $[M + 1]^+$; 91 $[CH_2\text{-}Ph]^+$.

(1S)[1-^{2}H]-1-Benzyloxy-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-ethane (5b)

The procedure described for 5a was used to prepare compound 5b from 3b, replacing the ethanol d_6 by non-deuterated ethanol. Compounds 5a and 5b have very similar ^{1}H-NMR, IR and mass spectra.

(1S)[1-^{3}H]-1-Benzyloxy-2-(2,5,5-trimethyl-1,3-dioxane-2-yl)-ethane (5c)

The preparation of 5c from 3c was identical to that described for 5b.

Ethyl (3RS)(5R)[5-^{2}H]-3-hydroxy-3-methyl-5- benzyloxy-pentanoate (6a)

5 ml of IN HCl were added to a round bottom flask containing 2.35 g (8.86 mmol) of compound 5a in 50 ml of methanol. After stirring at room temperature for 15 min, the reaction mixture was neutralized with 5% aqueous $NaHCO_3$, then evaporated to dryness. Flash chromatography with ethyl acetate hexane (1:2 v/v) gave quantitatively the corresponding deprotected ketone which was used for the subsequent step. Under argon atmosphere, at -78°C, 19.1 mmol of n-butyl lithium were added slowly into a round bottom flask containing 19.1 mmol of di-isopropylamine in 60

ml of THF. The magnetic stirring was continued for 15 min, then 19.1 mmol of ethyl acetate were added dropwise. After 30 min of additional stirring at -78°C, the above deprotected ketone intermediate (9.09 mmol) in 10 mL of THF was added slowly by means of an equalizing pressure dropping funnel. The reaction mixture was stirred at -78°C for 45 min more, then quenched with 4ml of water. The crude product was extracted with ethyl acetate, washed with dilute H_2SO_4 until neutral pH. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness. Flash chromatography with ethyl acetate - hexane (1:3) gave pure ester 6a. Yield: 93%.

^{1}H-NMR ($CDCl_3$) δ (ppm): 1.25 (t, J = 8Hz, 3H, CH_2-CH_3); 1.35 (s, 3H, CH_3); 1.95 (d,J = 7Hz, 2H, CH_2-CDH); 2.55 (s, 2H, CH_2-CO_2); 3.65 (t, J = 7Hz, 1H, CH_2-DCHO); 3.90 (s, 1H, OH); 4.20 (t, J =8Hz, 2H, CH_3-CH_2-CO_2) 4.55 (s, 2H, CH_2Ph); 7.35 (s, 5H, Ph). IR ($CHCl_3$) ν(cm^1): 700, 1720, 2120, 3000, 3500. MS: m/e = 268 [M + 1]$^+$; 250 [M-H_2O + 1]$^+$, 91 [Ph-CH_2]$^+$.

Ethyl (3RS)(5S)[5-^{2}H]-3-hydroxy-3-methyl-5-benzyloxy-pentanoate (6b) and ethyl (3RS)(5S)[5-^{3}H]-3-hydroxy-3-methyl-5-benzyloxy-pentanoate (6c)

Esters 6b and 6c were respectively prepared from 5b and 5c by the same procedure described for 6a. Their spectra (IR;^{1}H-NMR;MS) were similar.

(3RS)(5R)[5-^{2}H]-Mevalonolactone 7a

2.092 g (7.83 mmol) of ester 6a were dissolved in 80ml of methanol and 0,90 ml of 1N HCl was added, followed by 455 mg of 10% palladium on carbon. This mixture was stirred at room temperature overnight, while hydrogen was bubbled throughout a glass capillary. The catalyst was removed by filtration through a celite column, then the organic layer was neutralized with anion resin exchange 1X8 (OH-). Filtration was followed by evaporation to dryness. Flash chromatography of the crude product with ethyl acetate - hexane (2:1 v/v) gave pure mevalonolactone (7a). Yield: 86%. ^{1}H-NMR ($CDCl_3$) δ (ppm); 1.45 (s, 3H, CH_3); 1.95 (d, 2H, 4-H); 2.25 (s, 1H, OH); 2.70 (s, 2H, 2-H); 4.60 (m, 1H, 5-H); IR ($CHCl_3$) ν(cm^{-1}): 1725, 3000, 3580. MS: m/e = 132 [M + 1]+

(3RS)(5S)[5-^{2}H]-Mevalonolactone (7b) and (3RS)(5S)[5-^{3}H]-Mevalonolactone (7c)

Mevalonolactones 7b and 7c were obtained from esters 6b and 6c respectively, according to the same procedure described for 7a. Compounds 7a and 7b have very similar IR and MS spectra. The ^{1}H-NMR and ^{2}H-NMR of 7a and 7b are identical. In the ^{1}H-NMR, two groups of peaks are detected, one corresponding to 5-H axial (at 4.312 ppm) and one to 5-H-equatorial (at 4.571 ppm).

For the tritiated mevalonolactone 7c, its R_f and R_t (TLC and HPLC) were identical to an authentic sample of unlabeled mevalonolactone. The specific radioactivity of the tritiated mevalonolactone was: 20.5 µci/µmol.

Acknowledgment: We are grateful to the National Science and Engineering Research Council of Canada for support of this work.

REFERENCES

1. L.O. Zamir, "Biosynthesis of 3-Acetyldeoxynivalenol". 1986 in "Mycotoxins and Phycotoxins" Elsevier Press, Amsterdam, The Netherlands, p. 109-124. P.S. Steyn and R. Vleggaard (Eds).
2. L.O. Zamir, Y. Nadeau, C.D. Nguyen, K. Devor and F. Sauriol. 1987. J. Chem. Soc. Chem. Comm. 127-129.
3. L.O. Zamir, Y. Nadeau, C.D. Nguyen. 1987. J. of Labelled Compounds and Radiopharmaceuticals (in press).
4. J.W. Conforth, F.P. Ross and C. Wakselman, J. Chem. Soc., Perkin Trans, 1: 429, (1974).
5. D.E. Cane, P.P. Murthy, J. Am. Chem. Soc, 99: 8327, (1977).
6. L.O. Zamir, F. Sauriol and C.D. Nguyen. Tet. Letters 3059-3060 (1987).
7. J.B. Jones and H.M. Schwartz, Can. J. Chem., 59: 1574, (1981)
8. T.H. Chan, M.A. Brook and T. Chaly, Synthesis, 203, (1983).