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Synthesis of Tritiated 1α,25-Dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71)[1]

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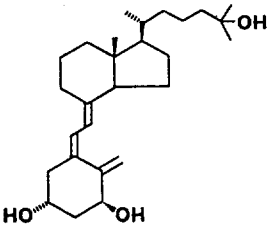
SUMMARY

The synthesis of tritiated 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71), [2β-(3-^{3}H)]ED-71 (**3**) and [26,27-$^3H_6$]ED-71 (**4**). is described. The former was prepared by reduction of a precausor containing a formyl group in the A-ring part with sodium borotritiide while the latter was labeled in the side chain using tritiated methylmagnesium iodide. The specific activity of **3** was 13.2Ci/mmol and that of **4** 138.0Ci/mmol.

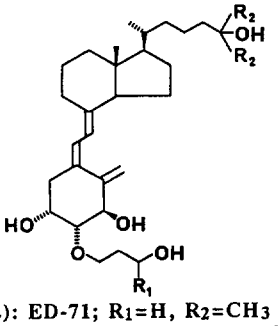
KEY WORDS: 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D_3 (ED-71), [2β-(3-^{3}H)]ED-71, [26,27-$^3H_6$]ED-71, sodium borotritiide, 1α,25-dihydroxyvitamin D_3

INTRODUCTION

Various analogs of 1α,25-dihydroxyvitamin D_3 [1α,25(OH)$_2$D$_3$] (**1**), a hormonally active form of vitamin D_3, have been synthesized in attempts to separate differentiation-induction and



(1): 1α,25(OH)$_2$D$_3$



(2): ED-71; R_1=H, R_2=CH_3
(3): [2β-(3-^{3}H)]ED-71; R_1=^{3}H, R_2=CH_3
(4): [26,27-$^3H_6$]ED-71; R_1=H, R_2=C^3H_3

Chart 1

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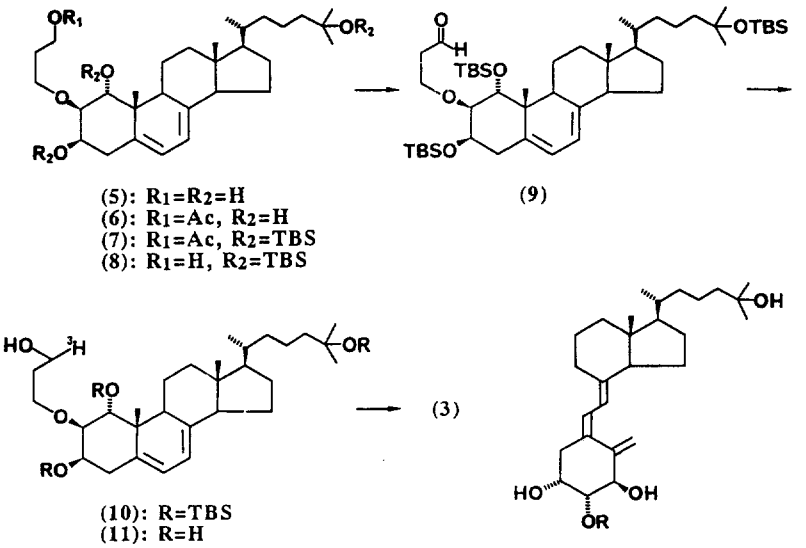
antiproliferation activities from calcemic activity, with the aim of obtaining useful analogs for the medical treatment of psoriasis, secondary hyperparathyroidism, cancer, immunological disorders, etc.[2] There is also an intense interest in obtaining compounds more active than 1α,25(OH)$_2$D$_3$ (1) in terms of regulatory effects on calcium and phosphorous metabolism, with the aim of treating bone diseases such as osteoporosis and osteopenia.[1,3-4] We have already reported that 1α,25-dihydroxy-2β-(3-hydroxypropoxy)vitamin D$_3$ (ED-71) (2) shows a significant increase in the bone mineral density of ovariectomized rats.[5] ED-71 (2) is now being clinically investigated as a candidate for the treatment of osteoporosis.[6]

During the course of our development of ED-71 (2), the synthesis of tritiated ED-71 was needed for pharmacokinetic and metabolic studies. In this paper we describe the synthesis of ED-71 tritiated at different positions, namely [2β-(3-^{3}H)]ED-71 (3) tritiated in the A-ring by reduction with sodium borotritiide (NaB[^{3}H$_4$]) and [26,27-^{3}H$_6$]ED-71 (4) labeled at the side chain with tritiated methylmagnesium iodide (C[^{3}H$_3$]MgI) (**Chart 1**).

SYNTHESIS

First, we undertook the synthesis of [2β-(3-^{3}H)]ED-71 (3). Introduction of tritium into the 2β-substituent was performed by NaB[^{3}H$_4$] reduction of the formyl group in the aldehyde (9), which was prepared from proED-71 (5)[3] by; i) acetylation of the primary hydroxy group giving 6, ii) silylation of the secondary and the tertiary hydroxy groups giving 7, iii) deacetylation giving 8, and iv) the modified Swern oxidation[7] giving 9. Thus, treatment of the aldehyde (9) with 1Ci of NaB[^{3}H$_4$] (46.1Ci/mmol) at room temperature gave 393mCi of the tritiated alcohol (10) in 94% yield, which was then desilylated by tetra-*n*-butylammonium fluoride (TBAF) to 237mCi of the



(5): R_1=R_2=H
(6): R_1=Ac, R_2=H
(7): R_1=Ac, R_2=TBS
(8): R_1=H, R_2=TBS

(9)

(10): R=TBS
(11): R=H

(12): R=H
(13): R=CH_2CH_2COOH

TBS=*tert*-Butyldimethylsilyl

Chart 2

tritiated proED-71 (**11**) in 60% yield. Subsequent irradiation of **11** with a high pressure mercury lamp through a Vycor filter, followed by thermal isomerization provided practically pure 72.5mCi of [2β-(3-^{3}H)]ED-71 (**3**) in 41% yield after preparative thin layer chromatography (TLC). The analytically pure **3** was obtained in 99% radiochemical purity by further reverse phase high-performance liquid chromatography (RP-HPLC); the specific activity was found to be 13.2Ci/mmol (27.0μCi/μg) (**Chart 2**).

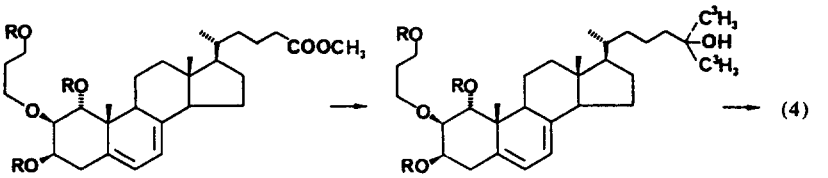
In the preliminary metabolic studies of ED-71 (**2**), the truncated metabolite (**12**) and the oxidized metabolite (**13**) at the site of the 2β-substituent of ED-71 (**2**), which lost the radioactivity of [2β-(3-^{3}H)]ED-71 (**3**), were observed (**Chart 2**). We, therefore, turned our attention to the synthesis of ED-71 labeled at the side chain, taking the metabolic loss of the tritiated part into consideration.

Next, we chose [26,27-$^3H_6$]ED-71 (**4**) as a target compound. To synthesize **4**, the ester (**14**) was prepared from lithocholic acid as described previously.[1] After protection of the hydroxy groups in **14** as silyl ethers (**15**), treatment of **15** with C[^{3}H$_3$]MgI, prepared from 19.4mg of magnesium and 50Ci of [^{3}H$_3$]methyl iodide (80Ci/mmol), gave 1.4Ci of the [26,27-$^3H_6$] silyl ether (**16**) with a radiochemical purity of 93-96% and a specific activity of 148Ci/mmol after HPLC purification. 1.4Ci of the silyl ether (**16**) was cleaved by TBAF to give 964mCi of [26,27-$^3H_6$]proED-71 (**17**) in 69% yield. Subsequent irradiation of **17** (964mCi) in ethanol with a high pressure mercury lamp through a Vycor filter, followed by thermal isomerization provided practically pure [26,27-$^3H_6$]ED-71 (**4**) (251mCi, 26%) after preparative TLC. The analytically pure **4** was obtained in 99% radiochemical purity by further purification using RP-HPLC; the specific activity was found to be 138.0Ci/mmol (274.4μCi/μg) (**Chart 3**).

Since the radioactivity of [26,27-$^3H_6$]ED-71 (**4**) is retained after *in vivo* administration, **4** has been used in pharmacokinetic and metabolic studies. The detailed results of pharmacokinetic and metabolic studies of ED-71 (**2**) using [2β-(3-^{3}H)]ED-71 (**3**) and [26,27-$^3H_6$]ED-71 (**4**) will be reported elsewhere.

EXPERIMENTAL

Melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Hitachi 270-30 spectrometer or JEOL JIR-6000 and proton nuclear magnetic resonance spectra (NMR) with a JEOL FX-200 or JEOL JNM-270EX in $CDCl_3$ with tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz.



(14): R=H
(15): R=TBS

(16): R=TBS
(17): R=H

Chart 3

Mass spectra (EI-MS) were obtained with a Shimadzu GCMS QP-1000, high-resolution mass spectra (HR-MS) with a VG Auto Spec Q, and ultra violet spectra (UV) with a Shimadzu UV-240. The apparatus used for HPLC was a Tosoh CCP with UV detector UV-8010 and RI detector RS-8000. RP-HPLC was carried out on a YMC ODS A-312 at a flow rate of 1ml/min with MeOH/H_2O (85:15). Normal phase-HPLC was carried out on a Shimadzu-Du Pont Zorbax SIL at a flow rate 1.8ml/min with CH_2Cl_2/MeOH (24:1). Radioactivity was measured with an Aloka LSC-900.

All reactions were carried out under an atmosphere of dry argon or nitrogen. Flash column chromatography was carried out with Merck Kiselgel 60, 230-400 mesh, and preparative TLC was performed on 20 x 20cm plates coated with a 0.25mm thickness of Merck Kiselgel 60 coating F_{254} indicator. NaB[$^3H_4$] was purchased from Amersham Japan (code No. TRK 838).

2β-(3-Acetoxypropoxy)-1α,3β,25-trihydroxycholesta-5,7-diene (6)

A mixture of 5 (312mg, 636μmol), pyridine (16.7ml), and Ac_2O (2.8ml) was stirred at 0°C for 2.5h. The mixture was poured into 3N HCl solution, extracted with AcOEt, washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (1:3) as the eluant to give 6 (225mg, 72%) as a colorless oil. IR (neat) 3430 (br), 2950, 1740, 1730, 1370, 1240cm^{-1}. NMR δ 0.62 (3H, s), 0.96 (3H, d, J=6.3Hz), 1.03 (3H, s), 1.21 (6H, s), 2.06 (3H, s), 3.48-3.59 (1H, m), 3.67-3.84 (3H, m), 3.89-4.00 (1H, m), 4.06-4.32 (2H, m), 5.31-5.38 (1H, m), 5.64-5.68 (1H, m). EI-MS (m/z) 532 (M^+), 101 (100%). UV λmax nm 293, 281, 270.

2β-(3-Acetoxypropoxy)-1α,3β,25-tris(*tert*-butyldimethylsilyloxy)cholesta-5,7-diene (7)

A mixture of 6 (260mg, 488μmol), TBSCl (1.47g, 9.75mmol), and imidazole (2.35g, 39.1mmol) in 1,3-dimethyl-2-imidazolidinone (DMI) (7ml) was stirred at 120°C for 18h. The mixture was diluted with *n*-hexane and washed with H_2O. The aqueous layer was extracted with AcOEt. The combined organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (93:7) as the eluant to give 7 (391mg, 92%) as a pale yellow oil. IR (neat) 2950, 2930, 2855, 1745, 1360, 1250, 1070, 830, 765cm^{-1}. NMR δ 0.04 (9H, s), 0.06 (3H, s), 0.09 (3H, s), 0.11 (3H, s), 0.60 (3H, s), 0.84 (9H, s), 0.87 (9H, s), 0.88 (9H, s), 0.93 (3H, d, J=6.1Hz), 1.01 (3H, s), 1.16 (6H, s), 2.02 (3H, s), 3.48-3.70 (1H, m), 3.77-3.90 (1H, m), 3.98-4.21 (3H, m), 5.22-5.31 (1H, m), 5.50-5.58 (1H, m). EI-MS (m/z) 874 (M^+), 147 (100%). UV λmax nm 293, 282, 270.

2β-(3-Hydroxypropoxy)-1α,3β,25-tris(*tert*-butyldimethylsilyloxy)cholesta-5,7-diene (8)

To a stirred mixture of $LiAlH_4$ (7.1mg, 187μmol) in THF (1ml), was added 7 (81mg, 92.5μmol) in THF (2ml) at 0°C. The resulting mixture was stirred at 0°C for 3h, quenched by 1N NaOH at 0°C, extracted with AcOEt, washed with saturated NaCl, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (9:1) as the eluant to give 8 (65mg, 84%) as a colorless oil. IR (neat) 3450 (br), 2945, 2920, 2850, 1470, 1250, 1080, 830, 765cm^{-1}. NMR δ 0.06 (6H, s), 0.08 (3H, s), 0.09 (3H, s), 0.11

(3H, s), 0.14 (3H, s), 0.62 (3H, s), 0.85 (9H, s), 0.90 (9H, s), 0.91 (9H, s), 0.95 (3H, d, J=6.2Hz), 1.05 (3H, s), 1.18 (6H, s), 3.59 (1H, brs), 3.67-3.96 (5H, m), 4.00-4.14 (1H, m), 5.21-5.35 (1H, m), 5.50-5.61 (1H, m). EI-MS (m/z) 832 (M^+), 301 (100%). UV λmax nm 293, 282, 271.

2β-(3-Oxopropoxy)-1α,3β,25-tris(*tert*-butyldimethylsilyloxy)cholesta-5,7-diene (9)

DMSO (26.4μl, 372μmol) was added to a stirred solution of triphosgene (19.2mg, 64.7μmol) in CH_2Cl_2 (0.5ml) at -60°C. The mixture was stirred for 5min and **8** (49mg, 58.8μmol) in CH_2Cl_2 (0.5ml) was added at -60°C. The stirring was continued for 15min at the same temperature, then triethylamine (61.8μl, 443μmol) was added and the mixture was stirred at the same temperature for 10min and at room temperature for 30min. The mixture was poured into H_2O, extracted with CH_2Cl_2, washed with saturated NaCl, dried over $MgSO_4$, and evaporated. The residue was purified by preparative TLC developed with *n*-hexane/AcOEt (9:1) to give **9** (22mg, 44%) as a colorless oil. IR (neat) 2950, 2930, 2855, 1735, 1460, 1365, 1250, 1090, 835, 770cm^{-1}. NMR δ 0.06 (9H, s), 0.08 (3H, s), 0.11 (3H, s), 0.15 (3H, s), 0.61 (3H, s), 0.85 (9H, s), 0.89 (9H, s), 0.90 (9H, s), 0.91 (3H, d, J=6.2Hz), 0.97 (3H, s), 1.18 (6H, s), 3.58 (1H, brs), 3.69 (1H, brd, J=3.9Hz), 3.84-4.20 (3H, m), 5.26-5.35 (1H, m), 5.52-5.62 (1H, m), 9.82 (1H, t, J=2.0Hz). EI-MS (m/z) 830 (M^+), 301 (100%). UV λmax nm 293, 282, 270.

2β-([3-^{3}H]-3-Hydroxypropoxy)-1α,3β,25-tris(*tert*-butyldimethylsilyloxy)-cholesta-5,7-diene (10)

To a stirred solution of **9** (30.0mg, 36.1μmol) in EtOH (600μl), was added a solution of Na$B[^3H_4]$ (1Ci, 46.1Ci/mmol) at room temperature. The resulting mixture was stirred at room temperature for 1.5h, quenched by addition of acetone, and concentrated *in vacuo*. The residue was purified by preparative TLC developed with AcOEt/*n*-hexane (1:9) to give **10** as a colorless oil (393mCi, 94%), which was identical with cold authentic **8** and used without further purification.

2β-([3-^{3}H]-3-Hydroxypropoxy)-1α,3β,25-trihydroxycholesta-5,7-diene (11)

A mixture of **10** (393mCi), TBAF (1M solution in THF, 5ml), and molecular sieves 4A (520mg) in DMI (1.7ml) was stirred at 100°C for 2h. The mixture was diluted with AcOEt (150ml), washed with saturated $NaHCO_3$ (50ml x 2) and H_2O (50ml x 1), dried over $MgSO_4$ and evaporated. The residue was purified by preparative TLC developed with CH_2Cl_2/EtOH (100:13) to give **11** as a colorless powder (237mCi, 60%), whose behavior on TLC and HPLC was identical with cold authentic **5**[3]: a colorless powder, mp118-120°C. IR (nujol) 3350, 1130, 1090, 1050, 1030cm^{-1}. NMR δ 0.62 (3H, s), 0.96 (3H, d, J=6.3Hz), 1.06 (3H, s), 1.22 (6H, s), 5.32-5.42 (1H, m), 5.64-5.73 (1H, m). EI-MS (m/z) 490 (M^+), 472, 454, 396, 131 (100%). UV λmax nm 293, 281.5, 271, 262 (shoulder). Anal. Calcd for $C_{30}H_{50}O_5$ 3/4H_2O: C, 71.46; H, 10.30. Found: C, 71.44; H, 9.87.

2β-([3-^{3}H]-3-Hydroxypropoxy)-1α,3β,25-trihydroxycholesta-5,7,10(19)-triene; [2β-(3-^{3}H)]ED-71 (3)

A solution of **11** (237mCi) in EtOH (200ml) was irradiated using a 400W high pressure mercury lamp with a Vycor filter at 0°C for 100sec. The mixture was refluxed for 2h and concentrated *in vacuo*. The residue was purified by preparative TLC developed with CH_2Cl_2/EtOH (50:7) to give practically pure **3** (72.5mCi, 31%) as a pale yellow oil and recovered **11** (115mCi, 49%). The above-mentioned irradiation, thermal isomerization and purification were repeated with the recovered **11** (115mCi) to give further **3** (23.9mCi, 10%). The analytically pure **3** was obtained by further purification of practically pure **3** using RP-HPLC in 99% radiochemical purity with specific activity of 13.2Ci/mmol (27.0mCi/mg). The behavior of **3** on TLC and HPLC was identical with cold authentic **2**[3]: a colorless foam. IR (nujol) 3360, 1100, 1060, 910cm^{-1}. NMR δ 0.55 (3H, s), 0.91 (3H, d, J=6.1Hz), 1.21 (6H, s), 3.60-4.02 (5H, br), 4.12-4.36 (2H, m), 5.08 (1H, s), 5.49 (1H, s), 6.04 (1H, d, J=10.5Hz), 6.36 (1H, d, J=10.5Hz). EI-MS 490 (M^+), 472, 454, 396, 59 (100%). HR-MS Calcd for $C_{30}H_{50}O_5$: 490.3658. Found: 490.3678. UV λmax nm 263.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-2β-(3-*tert*-butyldimethylsilyloxy-propoxy)-20(*R*)-(3-methoxycarbonylpropyl)pregna-5,7-diene (15)

A mixture of **14** (348mg, 710μmol), TBSOTf (2.19ml, 9.54mmol), and 2,6-lutidine (1.85ml, 15.9mmol) in CH_2Cl_2 (30ml) was stirred at room temperature for 1.5h. The mixture was poured into 1N HCl, extracted with CH_2Cl_2, washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography with *n*-hexane/AcOEt (98:2) as the eluant to give **15** (541mg, 92%) as a colorless oil. IR (neat) 2950, 2925, 2850, 1745, 1470, 1385, 1255, 1090, 835cm^{-1}. NMR δ 0.05 (9H, s), 0.07 (3H, s), 0.10 (3H, s), 0.13 (3H, s), 0.62 (3H, s), 0.89 (27H, s), 0.96 (3H, d, J=6.3Hz), 1.03 (3H, s), 3.49-4.12 (7H, m), 3.67 (3H, s), 5.25-5.37 (1H, m), 5.52-5.65 (1H, m). EI-MS (m/z) 832 (M^+), 73 (100%). UV λmax nm 293, 281, 271.

1α,3β-Bis(*tert*-butyldimethylsilyloxy)-2β-(3-*tert*-butyldimethylsilyloxy-propoxy)-25-hydroxy-[26,27-^{3}H$_6$]cholesta-5,7-diene (16)

To a mixture of Mg powder (19.4mg, 798μmol) and I_2 (2.0mg) in Et_2O (5ml) was distilled C[^{3}H$_3$]I (50Ci, 80Ci/mmol, 0.625mmol) under liquid N_2 cooling. The resulting mixture was warmed to ambient temperature and stood for 10min, when effervescence was observed, then heated at 30°C for 45min. The mixture was then cooled to -15°C and **15** (50mg, 60mmol) in Et_2O (5ml) was added. The resulting mixture was stirred at -15°C for 1h, and then further at 0°C for 1h, quenched by addition of saturated NH_4Cl (15ml), extracted with CH_2Cl_2, dried over $MgSO_4$ and evaporated to give crude **16** (4.9Ci). Crude **16** was purified by RP-HPLC to give **16** (1.4Ci) in 93-96% radiochemical purity with specific activity of 148Ci/mmol. The behavior of **16** on TLC and HPLC was identical with cold **16**: a colorless oil. IR (neat) 3370 (br), 2950, 2930, 2855, 1470, 1255, 1090, 835, 775cm^{-1}. NMR δ 0.05 (9H, s), 0.07 (3H, s), 0.10 (3H, s), 0.13 (3H, s), 0.62 (3H, s), 0.89 (27H, s), 0.96 (3H, d, J=6.3Hz), 1.03 (3H, s), 1.22 (6H, s), 3.50-4.14 (7H, m), 5.25-5.34 (1H, m), 5.52-5.64 (1H, m). EI-MS 832 (M^+), 73 (100%). UV λmax nm 293, 282, 271.

2β-(3-Hydroxypropoxy)-1α,3β,25-trihydroxy-[26,27-$^3H_6$]cholesta-5,7-diene (17)

A mixture of **16** (1.4Ci, 148Ci/mmol, 9.5μmol) and TBAF (1M in THF, 300μl, 300μmol) in THF (3ml) was stirred at 65°C for 15h. The mixture was diluted with AcOEt, washed with saturated $NaHCO_3$, dried over $MgSO_4$ and evaporated. The residue was purified by preparative TLC developed with CH_2Cl_2/EtOH (100:13) to give **17** (964mCi, 69%) as a colorless powder. The behavior of **17** on TLC and HPLC was identical with cold authentic **5**[3], as described in the synthesis of **11**.

2β-(3-Hydroxypropoxy)-1α,3β,25-trihydroxy-9,10-seco-[26,27-$^3H_6$]-5,7,10(19)-triene; [26,27-$^3H_6$]ED-71 (4)

A solution of **17** (964mCi, 6.5μmol) in EtOH (200ml) was irradiated using a 400W high pressure mercury lamp with a Vycor filter at 0°C for 80sec. The mixture was refluxed for 2h and concentrated *in vacuo*. The residue was purified by preparative TLC developed with CH_2Cl_2/EtOH (100:13) to give practically pure **4** (164mCi, 17%) as a pale yellow oil and recovered **17** (536mCi, 56%). The above-mentioned irradiation, thermal isomerization and purification were repeated with the recovered **17** (536mCi) to give further **4** (87mCi, 9%). The analytically pure **4** was obtained by further purification of practically pure **4** using RP-HPLC in 99% radiochemical purity with specific activity of 138.0Ci/mmol (274.4mCi/mg). The behavior of **4** on TLC and HPLC was identical with cold authentic **2**[3] as described in the synthesis of **3**.

ACKNOWLEDGMENT

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