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The Synthesis of a Phorbol 12,13-Bis-lactone

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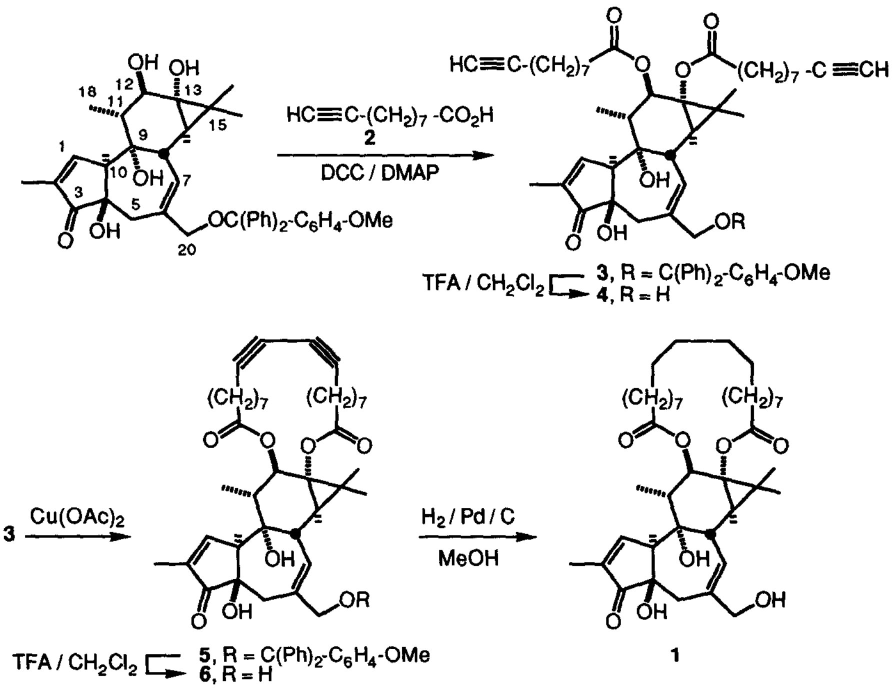
Abstract: *The synthesis of a phorbol derivative (1) containing a 24-membered ring 12,13-bis-lactone was achieved via bis-acylation of 20-methoxytritylphorbol with decynoic acid, followed by a copper mediated macrocyclic ring closure, deprotection and selective reduction.*

Since the various isoforms of protein kinase C (PKC) are known mediators of many intracellular responses to extracellular signals,[2] we have been interested in the synthesis of modulators of PKC activity, that may have the potential for isozyme selective agonist or antagonist activity.[3] There has been considerable interest in diacyglycerol anologues that mediate the activity of PKC,[4] and Wender and Cribbs have reported a series of cyclic diacylglycerol mimics with greater potency as PKC agonists than simple diacylglycerols.[5] We considered it of interest to incorporate Wender's bis-lactone concept onto the phorbol nucleus, since it is well accepted that diacyl glycerols are the natural agonists that the more potent phorbol diesters replace.[2b] This has been achieved by a reaction sequence analogous to that reported by the Wender group[5] (Scheme 1).



Scheme 1

Addition of lithium acetylide·ethylenediamine complex to 8-bromooctanoic acid in DMSO gave 9-decynoic acid (**2**) in 94% isolated yield.[6] Bis-acylation of 20-methoxytritylphorbol[7] with (**2**) (DCC / cat. DMAP in CH_2Cl_2 / Et_2O, 18 h), gave the diester (**3**) as a single product (tlc) quantitatively, after a silica filtration (95:3, CH_2Cl_2 / Et_2O). Deprotection of (**3**) by mild acid treatment (100 mg of (**3**) in 100 µL TFA / 5 mL CH_2Cl_2) gave a quantitative yield of 20-hydroxyl-12,13-bis-decynoate (**4**),[8] after flash silica chromatography (55:45, EtOAc / hexane, Rf = 0.4). Copper (II)-mediated coupling of (**3**) to give the macrocyclic product (**5**) was achieved in pyridine / ether,[9] and subjection of the crude product to acid catalyzed deprotection furnished the 20-hydroxyphorbol (**6**). Selective reduction of the diyne was accomplished by atmospheric pressure hydrogenation (balloon) over a 10% Pd / C catalyst in methanol for 30 minutes at room temperature, while monitoring the reaction by tlc. Flash chromatography (1:1, EtOAc / hexane) gave the title compound (**1**) in 56% yield.[10]

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   CAUTION: All phorbol ester derivatives are potentially potent co-carcinogens and should be handled accordingly.
8. υ_{max} (liq. film) 3409, 3309, 2930, 2857, 2114, 1735, 1715, 1629 cm^{-1}, δ_H (CD_3COCD_3, 300MHz) 1.67 (3H, m, 19-CH_3), 2.50 (2H, m, 5-CH_2), 3.15 (1H, m, 10-CH), 3.27 (1H, m, 8-CH), 3.83 (1H, m, exchangeable, 20-OH), 3.92 (2H, m, 20-CH_2), 4.80 (1H, s, exchangeable, OH), 5.28 (1H, s, exchangeable, OH), 5.45 (1H, d, J = 10 Hz, 12-CH), 5.60 (1H, d, J = 6 Hz, 7-CH), 7.51 (1H, m, 1-CH). Found (FAB ms): 665.4050, calc.for $C_{40}H_{57}O_8$ $(MH)^+$ requires 665.4053.
9. In a typical procedure, the bis-decynoate (**3**) (500 mg, 0.54 mmol) in ether (20 mL) was added over 1h to a stirred solution of cupric acetate (540 mg, 2.7 mmol) in pyridine / ether (2:1, 500 mL) at 100°C (reflux), during which time the reaction turned from blue to green. After a further hour at 100°C the solvent was removed by evaporation. The residue was partitioned between 1:1 ethyl acetate / hexane and water, the organic phase washed with pH 2 phosphate buffer, saturated bicarbonate, brine, dried (Na_2SO_4), and the solvent evaporated to give (**5**). The crude product was deprotected by dissolution in dichloromethane (30 mL), and the dropwise addition of trifluoroacetic acid (300 µL). After 10 minutes tlc indicated complete reaction, the solution was washed with saturated bicarbonate, dried (Na_2SO_4), and the solvent evaporated. Chromatography on silica gel eluted with 45:55 ethyl acetate / hexane gave (**6**), 236 mg, 66%, υ_{max} (liq. film) 3409, 2930, 2857, 2260 (w), 2167 (w), 1735, 1709 cm^{-1}, δ_H (CD_3COCD_3) 1.50 (4H, m, $CH_2C\equiv$), 1.67 (3H, m, 19-CH_3), 2.52 (2H, m, 5-CH_2), 3.15 (1H, m, 8-CH), 3.27 (1H, m, 10-CH), 3.83 (1H, m, exchangeable, 20-OH), 3.95 (2H, m, 20-CH_2), 4.80 (1H, s, exchangeable, OH), 5.28 (1H, s, exchangeable, OH), 5.48 (1H, d, J = 10 Hz, 12-CH), 5.61 (1H, d, J = 6 Hz, 7-CH), 7.52 (1H, m, 1-CH), Found (FAB ms): 663.3895, calc.for $C_{40}H_{55}O_8$ $(MH)^+$ requires 663.3897.
10. υ_{max} (liq. film) 3415, 2924, 2857, 1735, 1715, 1629 (w), cm^{-1}, δ_H (CD_3COCD_3) 1.67 (3H, m, 19-CH_3), 2.50 (2H, m, 5-CH_2), 3.15 (1H, m, 8-CH), 3.29 (1H, m, 10-CH), 3.83 (1H, m, exchangeable, 20-OH), 3.95 (2H, m, 20-CH_2), 4.80 (1H, s, exchangeable, OH), 5.27 (1H, s, exchangeable, OH), 5.50 (1H, d, J = 10 Hz, 12-CH), 5.62 (1H, d, J = 6 Hz, 7-CH), 7.52 (1H, m, 1-CH), Found (FAB ms): 671.4525, calc.for $C_{40}H_{63}O_8$ $(MH)^+$ requires 671.4523.