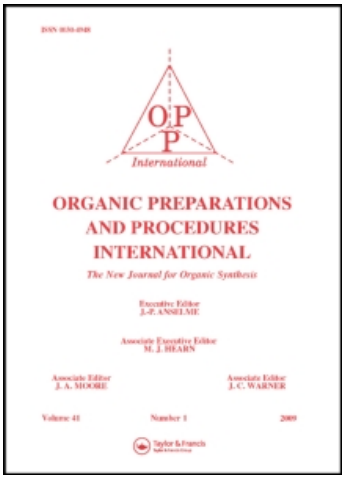


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# ALLYLIC AND PHENOLIC PHOSPHATE ESTERS OF DEXANABINOL

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## ALLYLIC AND PHENOLIC PHOSPHATE ESTERS OF DEXANABINOL

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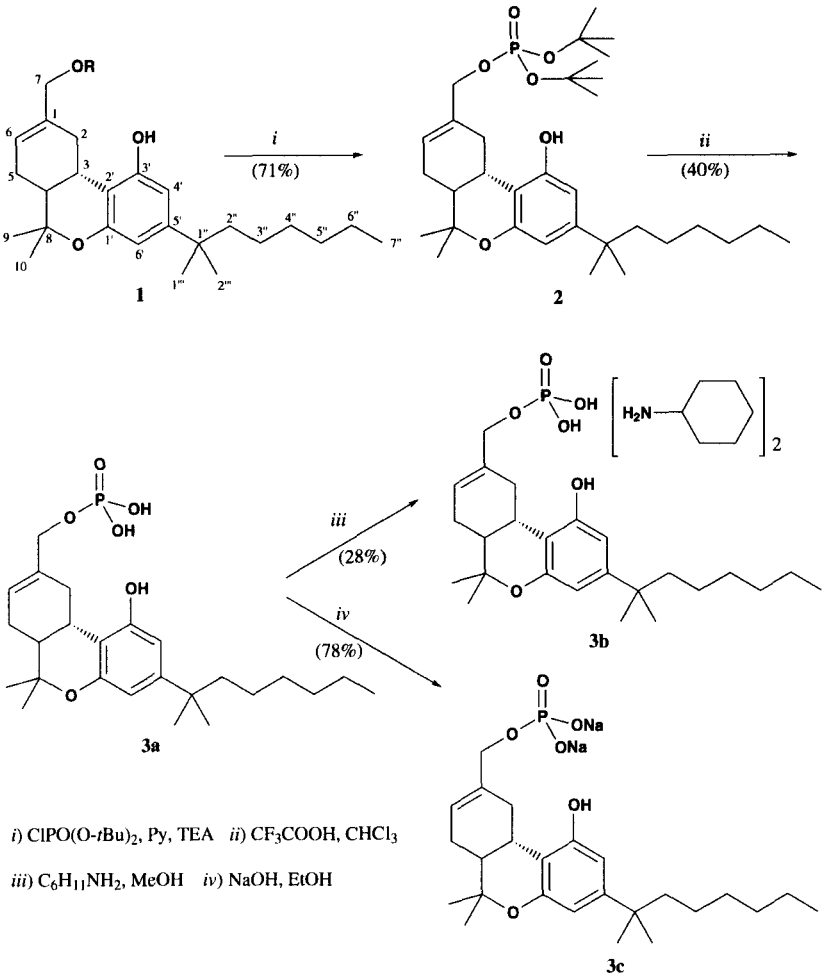
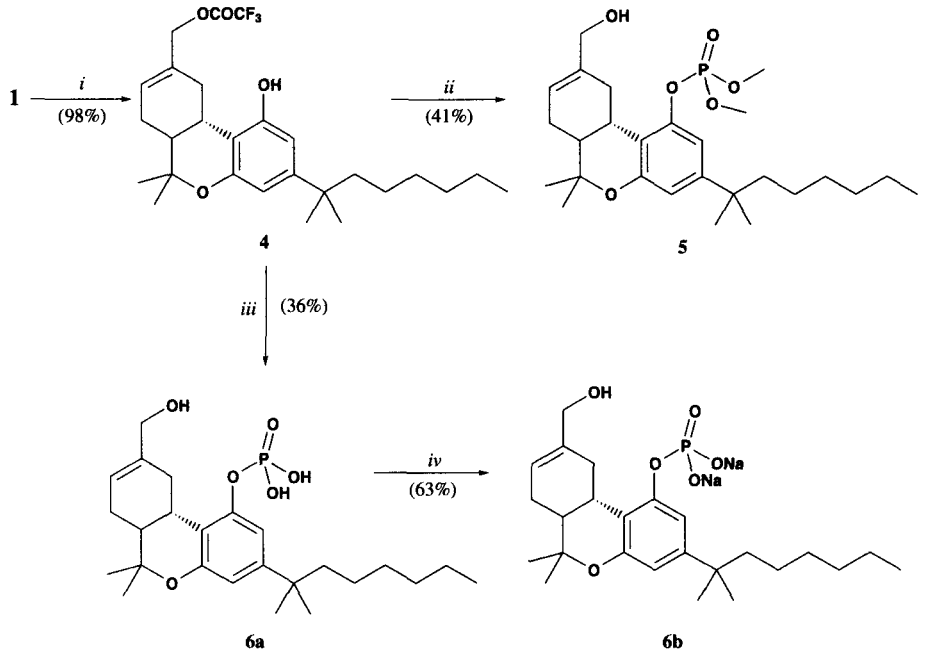
While natural cannabinoids belonging to the (-) *3R, 4R* series bind to specific central and peripheral receptors,[1] the synthetic (+) *3S, 4S* epimers, of which the 5'(1',1'-dimethylheptyl)-7-hydroxy-$\Delta^6$-tetrahydrocannabinol (dexanabinol, HU-211) (**1**) is the benchmark compound, are devoid of any cannabimimetic activity, since they have only negligible affinity to these receptors.[2] On the other hand, **1** proved to be a noncompetitive N-methyl-D-aspartate (NMDA) inhibitor [3] and an effective scavenger of peroxy and hydroxy radicals [4] and has been evaluated as a neuroprotective agent.[5-8] The further development of **1** as a therapeutic agent is somewhat hampered by its very low solubility in water, which complicates the formulation for intravenous administration. Various water-soluble esters of **1** containing polar or permanent charge-bearing moieties [9] including the glycinate salts [10] and salts of amino acid esters containing cyclic nitrogen [11] were evaluated as possible prodrugs or congeners. The syntheses of two phosphate esters of **1** are described herein.

Attempts to synthesize the allylic phosphate **3a** by reaction of dexanabinol with phosphorus oxychloride ($POCl_3$) and pyrophosphoryl chloride under various conditions, with or without base failed, since the 7-chloro derivative or a mixture of various unwanted products (dimers, polymers etc.) resulted. The reaction of **1** with freshly prepared di-*tert*-butylphosphorochloridate [12] in pyridine in the presence of triethylamine at -20° resulted in the 7-(di-*tert*-butyl) phosphate (**2**) which was purified by chromatography; the structure of **2** was unambiguously proven by $^1H$ and $^{13}C$ NMR spectroscopy. Reaction of **2** with trifluoroacetic acid in chloroform at room temperature for 30 min., the 7-dihydrogen phosphate **3a** resulted (structure proved by NMR and high resolution mass spectrometry). The *bis* cyclohexylammonium salt **3b** prepared from **3a** and cyclohexylamine in dry methanol and purified by recrystallization from *I*-propanol was used for characterization of the allylic phosphate. The sodium salt **3c** prepared from **3a** and ethanolic NaOH was used for solubility and stability studies (Scheme I).




The phenolic phosphate **6a** was synthesized according to the procedure described in Scheme II.

The allylic hydroxyl group was protected by preparing the trifluoroacetate **4**, using previously reported procedures.[13] Reaction of **4** with phosphorus oxychloride in pyridine followed by the treatment of the resulting protected phenolic phosphate with methanol gave the dimethyl phosphate **5**; this compound was prepared to establish the structure of the phenolic phosphate. If the protected phosphate was treated with aqueous $NaHCO_3$ (by simply washing the organic solution) the dihydrogen phosphate **6a** resulted as an oil (after column chromatography). The sodium salt **6b** was obtained by reaction of **6a** with ethanolic NaOH. Aqueous solutions (~2 %) of **3c** and **6b** were prepared by dissolving the crude salts in deionized water. After filtration the clear solutions were freeze-dried to afford the soluble salts used for further studies.




*i*) $CF_3COOH$ *ii*) a) $POCl_3$, Py b) MeOH *iii*) $POCl_3$, Py b) $NaHCO_3$ *iv*) NaOH, EtOH

## EXPERIMENTAL SECTION

Melting points are uncorrected and were determined on an Electro-thermal melting point apparatus (Fisher Scientific). Elemental micro combustion analyses were performed by Atlantic Microlabs Inc., Atlanta, GA. Proton ($^1$H NMR) and carbon ($^{13}$C NMR) nuclear magnetic resonance spectra were recorded on a Varian XL-300 spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts were reported as parts per million (δ) relative to TMS (0.00) which served as an internal standard. Coupling constants (J) are reported in Hertz. High resolution mass spectrometry (MS) was performed using a Finnigan MAT95Q instrument. The fast atom bombardment (FAB) technique was used; the matrix used was glycerine/trifluoroacetic acid (GLY/TFA). Thin layer chromatography was performed on EM reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel (60 mesh). All solvents and chemicals were reagent grade. Dexanabinol was synthesized in-house.

**Dexanabinol 7-O-(di-*t*-Butyl)phosphate (2).**- To a solution of dexanabinol (3.22 g, 8.33 mmol) in a mixture of dry pyridine (16 mL) and dry triethylamine (3.40 g, 25.0 mmol) was added as a solution of freshly prepared di-*tert*-butylphosphorochloridate (5.72 g. 25.0 mmol) in dry methylene chloride (80 mL) at -40° and the resulting solution was stored at -15° for 3 days. The solvent was removed *in vacuo* (1 mm Hg) and the residue was dissolved in methylene chloride (75 mL), the resulting solution was washed with 5% aqueous $NaHCO_3$ (40 mL) and brine (40 mL), dried ($MgSO_4$) and evaporated (1 mm Hg, 20-25°). The residue (3.85 g) was chromatographed (silica gel: 500 g, eluent: methylene chloride:methanol, 95:55 v/v) to provide 3.41 g (71%) of pure **2** as a pale yellow viscous oil. $R_f$ ($CH_2Cl_2$: MeOH, 95:5 v/v): 0.54.

$^1$H NMR ($CDCl_3$): δ 0.84 (3H, t, $J_{7'',6''}$ = 6.8 Hz, C7''-$H_3$), 1.00-1.35 (m, including 2 singlets at 1.10 [C10-$H_3$] and 1.19 [C1'''-$H_3$, C2'''-$H_3$), 1.38 (3H, s, C9-$H_3$), 1.43-1.62 (m, including 2 singlets at 1.498 *and* 1.503, [2x O-C)$CH_3$)$_3$]), 1.76-1.95 (3H, m, C3-$H_\beta$, C4-H, C5-$H_\alpha$), 2.15-2.29 (1H, m, C5-$H_\beta$), 2.66-2.78 (1H, m, C3-H), 3.50 (1H, ss, $^2J_{2\alpha,2\beta}$ = 16.2 Hz, $^3J_{3,2\alpha}$ = 4.2 Hz, C2-Hα), 4.30-4.44 (2H, m, C7-$H_2$), 5.75-5.81 (1H, m, C6-H), 6.31 (1H, d, $J_{4',6'}$ = 1.7 Hz, C4'-H), 6.45 (1H, br s, C6'-H); $^{13}$C NMR ($CDCl_3$): 14.07 (C7''), 18.40 (C10), 22.65 (C6''), 24.60 (C5''), 27.58 (C9), 27.71 (C5), 28.61 and 28.69 (C1''' and C2'''), 29.85 and 29.91 (2 x O-C($\underline{C}H_3$)$_3$), 30.04 (C4''), *31.25 (C2), *31.51 (C3), 31.78 (C3''), 37.26 (C1''), 44.55 (C2''), 44.89 (C4), 70.54 and 70.62 (C7), 76.18 (C8), 82.65 and 82.70 (2x O-$\underline{C}$($CH_3$)$_3$), 105.74 (C4'), 106.51 (C6'), 109.55 (C2'), 123.16 (C6), 134.33 and 134.44 (C1), 149.73 (C5'), 154.01 and 156.05 (C1; and C3').

*Anal.* Calcd for $C_{33}H_{55}PO_6$: C, 68.48; H, 9.58. Found: C, 68.28; H, 9.53

**Dexanabinol 7-O-Dihydrogen Phosphate (3a)**. To a solution of **2** (1.45 g, 2.5 mmol) in dry chloroform (40 mL) was added trifluroracetic acid (1.93 mL, 25 mmol) and the resulting mixture was stirred for 40 min at 20-25°. The resulting solution was diluted with chloroform (20 mL), washed with 5% aqueous $NaHCO_3$ saturated with NaCl (50 mL) and brine (2 x 25 mL) dried (anhydrous $MgSO_4$), and evaporated *in vacuo* (1 mm Hg) at 20-25°. The residue (1.045 g) (crude phosphate) was chromatographed twice (silica gel, 100 g; eluent, methylene acetate:acetic acid:water, 85:10:5 v/v). The resulting material was dissolved in chloroform (25 mL), the remaining solution washed with 5% aqueous $NaHCO_3$ saturated with NaCl (2 X 25 mL) and brine (25 mL), dried ($MgSO_4$) and evaporated *in vacuo* (1mm Hg) at 20-25° to give 0.171 g of pure **3a** as a glassy material. M.S. (FAB-GLY/TFA) $[M + H]^+$ (m/z): 467. $^1$H NMR ($CDCl_3$ + $CF_3COOD$) broad but characteristic signals: δ 0.84 (C7''-$H_3$), 1.07 (C10-$H_3$), 1.18 (C1'''-$H_3$, C2'''-$H_3$), 1.43 (C9-$H_3$), 1.86 (C2-$H_\beta$, C4-H, C5-$H_\alpha$), 2.23 (C5-$H_\beta$), 2.63 (C3-H), 3.32 (C3-$H_\alpha$), 4.43 (C7-$H_2$), 5.81 (C6-H), 6.38 (C4'-H), 6.49 (C6-H).

*Anal.* Calcd for $C_{25}H_{39}O_6P•H_2O$: C, 61.97; H, 8.53. Found: C, 61.99; H, 8.25

**Dexanabinol 7-O-Dihydrogen Phosphate, *bis*-Cyclohexylammonium Salt (3b)**.- To a solution of **3a** (crude material, before column chromatography) (0.97 g, 2.1 mmol) in dry methanol (15 mL) was added cyclohexylamine (0.48 mL, 4.2 mmol) and the solution was evaporated to 5 mL volume. The salt from the concentrated solution slowly crystallized (10 days at 0°). The crystals were filtered, rinsed with dry methanol (4 x 2 mL) and dried *in vacuo* (1 mm Hg) at 20-25° to provide 0.467 g of **3b**. By recrystallization from *I*-propanol (6 mL) pure **3b** resulted (0.349 g, 28% yield) as white needles, mp. 176-180°.

*Anal.* Calcd for $C_{37}H_{65}N_2O_6P$: C, 66.84; H, 9.85; N, 4.21. Found: C, 66.62; H, 9.84; N, 4.17

**Dexanabinol 7-O-Dihydrogen Phosphate, Disodium Salt (3c)**.- To a solution of **3a** (0.052 g, 0.11 mmol) in dry ethanol (2 mL) was added a 0.268 M sodium hydroxide anhydrous ethanolic solution (0.84 mL, 0.22 mmol) at 20-25° (pH: 7-8). The solution was evaporated *in vacuo* (1 mm Hg) at 20-25°. The residue (0.055 g) was sonicated (3 hrs) with deionized water (under argon) (40 mL), the resulting colloidal solution filtered, rinsed with water (2 x 20 mL) and the clear solution freeze-dried affording **3c** (0.044 g, 78% yield) as a hygroscopic white solid, mp. > 200° (dec.).

**Dexanabinol 3'-O-Dimethyl Phosphate (5).**- To a solution of dexanabinol-7-trifluoroacetate (**4**) (obtained from **1** and trifluoroacetic acid) (0.231 g, 0.48 mmol) in dry pyridine (1 mL) was added freshly distilled phosphorous oxychloride (0.075 mL, 0.80 mmol) at ice-cooling and the mixture was stirred at 3-5° for 20 hrs. Dry methanol (0.20 mL, 5 mmol) was added to the solution (exothermic reaction, temperature raised from 20 to 50°), and the resulting mixture was stored at 20-25° for 5 hrs then the solvent was evaporated *in vacuo* (1 mm Hg) at 20-25°; toluene (3 mL) was added to the residue and then distilled to remove traces of pyridine. To the mixture was added dry toluene (3 mL), the suspension was filtered and rinsed with toluene (3 x 1 mL) then the combined solutions were evaporated *in vacuo* (1 mm Hg) at 20-25°. The residue (0.328 g) was chromatographed (silica gel, 25 g; eluent, acetonitrile:methylene chloride, 7:3, v/v) to provide pure **5** (0.098 g, 41% yield) as a light brown thick oil. Rf (acetonitrile: methylene chloride, 7:3, v/v): 0.61. $^1$H NMR ($CDCl_3$): δ 0.84 (3H, t, $^3J_{7'',6''}$ = 6.6 Hz, C7''-$H_3$), 0.95-1.35 (m, including 2 singlets at 1.12 [C10-$H_3$] and 1,23 [C1'''-$H_3$, C2'''-$H_3$]), 1.40 (3H, s, C9-$H_3$), 1.48-1.57 (2H, m), 1.78-1.98 (3H, m , C2-$H_\beta$, C4-H, C5-$H_\alpha$), 2.01 ($CH_3CN$), 2.15-2.30 (2H, m, C5-$H_\beta$), 2.83 (1H, td, $^3J_{3,2\beta}$ = 10.6 Hz, $^3J_{3,2\alpha}$ = 4.6 Hz and $^3J_{3,4}$ = 10.6 Hz, C3-H), 3.22 (1H, dd, $^2J_{2\alpha,2\beta}$ , = 15.8 Hz, C2-$H_\alpha$), 3.81, 3.82, 3.85 and 3.86 (6H, s, P-O-$CH_3$), 4.03 (2H, s, C7-$H_2$), 5.74 (1H, d, *J* = 4.6 Hz, C6-H), 6.61-6.65 (1H, m, C4'-H), 6.77-6.81 (1H, m, C6'-H); $^{13}$C NMR ($CDCl_3$): 14.22 (C7''), 18.55 (C10), 22.79 (C6''), 24.74 (C5''), 27.57 (C9), 27.66 (C5), 28.78 (C1''', C2'''), 30.10 (C4''), 31.55 (C3), *31.81 (C3''), *31.91 (C2), 37.70 (C1''), 44.48 (C2''), 45.11 (C4), 54.92, 55.04, 55.07 and 55.12 (P-O-$CH_3$), 66.90 (C7), 77.00 (C8), 109.98 (C4'), 112.40 (C6'), 114.50 and 114.59 (C2'), 121.13 (C6), 138.15 (C1), 149.67 and 149.76 (C3'), 150.47 (C5'), 154.46 (C1').

*Anal.* Calcd for $C_{27}H_{43}O_6P$ + 9% $CH_3CN$: C, 64.93; H, 8.63; N, 0.31. Found: C, 64.93; H, 8.86; N, 0.31

**Dexanabinol 3'-O-Dihydrogen Phosphate (6a).**- To a solution of **4** (0.405 g, 0.84 mmol) in dry pyridine (2 mL) was added freshly distilled phosphorous oxychloride (0.14 mL, 1.5 mmol) at -20° and the mixture was stirred at 3-5° for 20 hrs. The solvent was evaporated *in vacuo* (1mm Hg) at 20-25° and the residue was extracted with dry toluene (3 x 3 mL), the combined solutions were then evaporated *in vacuo* (1 mm Hg) at 20-25°. The residue was extracted with dry ether (2 x 10 mL) and the solution evaporated *in vacuo* (1 mm Hg) at 20-25°. The residue (0.485 g) was dissolved in dry ether (50 mL) and deionized water (30 mL) was added with ice cooling after which the mixture was stirred for 18 hrs at 0-5°. To the mixture brine (20 mL) was added, the layers were separated and the organic solution was washed with brine (3 x 10 mL) dried ($MgSO_4$) and evaporated *in vacuo* (1 mm Hg) at 20-25°. The residue was dissolved in ether (20 mL), followed by addition of 5% aqueous $NaHCO_3$ and the mixture was stirred for 16 hrs at 20-25°. The layers were separated, the organic solution was washed with brine (3 x 10 mL), dried ($MgSO_4$) and evaporated *in vacuo* (1 mm Hg, 20-25°). The residue (0.309 g) was chromatographed (silica gel: 25 g, eluent, methyl acetate: acetic acid: water, 85:10:5, v/v). Combined fractions were evaporated, the residue was dissolved in ether (10 mL), the solution was washed with 5% aqueous $NaHCO_3$ (3 x 5 mL) and brine (3 x 5 mL), dried ($MgSO_4$) and evaporated *in vacuo* (1 mm Hg, 20-25°) to provide pure **6a** (0.141 g, 36%) as a thick oil. Rf (methyl acetate:acetic acid:water, 85:10:5, v/v): 0.43. $^1$H NMR ($CDCl_3$ + $CF_3COOD$): δ 0.82 (3H, t, $^3J_{7'',6''}$ =

6.6 Hz, C7"-$H_3$), 0.92-1.29 (m, including 2 singlets at 1.02 [C10-$H_3$] and 1,16 (C1'"-$H_3$, C2'"-$H_3$]), 1.35 (3H, s, C9-$H_3$), 1.42-1.53 (2H, m), 1.60-1.93 (3H, m, C2-$H_\beta$, C4-H, C5-$H_\alpha$), 1.95-2.25 (1H, m, C5-$H_\beta$), 2.55-2.84 (1H, m, C3-H), 3.28-3.55 (1H, m, C2-$H_\alpha$), 3.78-4.10 (2H, m, C7-$H_2$), 5.66 (1H, br s, C6-H), 6.61 (1H, br s, C4'-H), 6.74 (1H, br s, C6'-H); $^{13}C$ NMR ($CDCl_3$ + $CF_3COOD$): δ 13.99 (C7"), 18.06 (C10), 22.62 (C6"), 24.50 (C5"), 27.34 (C9), 27.51 (C5), 28.28 (C1'" and C2'"), 29.92 (C4"), *30.69 (C3), *31.33 (C2), *31.72 (C3"), 37.42 (C1"), #44.28 (C2"), #44.50(C4), 67.11 (C7), 76.77 (C8), 110.32 (C4'), 112.21 (C6'), 114.27 (C2'), 125.02 (C6), 136.06 (C1), 149.91 (C3'), 150.66 (C5'), 154.66 (C1').

*Anal.* Calcd for $C_{25}H_{39}O_6P•H_2O$: C, 61.97; H, 8.53. Found: C, 62.05; H, 8.34

**Dexanabinol 3'-O-Dihydrogen Phosphate, Disodium Salt (6b).**- To a solution of **6a** (0.047 g, 0.10 mmol) in dry ethanol (5 mL) was added a 0.187 M sodium hydroxide solution in dry ethanol (1.07 mL, 0.20 mmol) under argon (pH ~8). The solvent was evaporated *in vacuo* (1 mm Hg, 20-25°) and the residue was sonicated with deionized water (20 mL) under argon for 2 hrs. The resulting colloidal solution was filtered, the filtrate rinsed with water (2 x 10 mL) and the clear solution was freeze-dried to afford **6b** (0.032 g, 63%) as a hygroscopic solid.



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## SYNTHESIS OF 4*H*-CYCLOPENTA[*def*]CHRYSENE AND OTHER METHYLENE-BRIDGED POLYCYCLIC HYDROCARBONS

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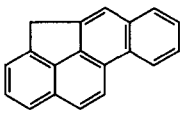
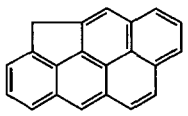
Methylene-bridged polycyclic aromatic hydrocarbons (PAHs) are widespread environmental contaminants formed in the incomplete combustion of fossil fuels.[1-3] Some bridged PAHs, notably 4*H*-cyclopenta[*def*]chrysene and 4*H*-benz[*def*]cyclopenta[*mno*]chrysene (**1** and **2**), exhibit significant activity as mutagens and carcinogens.[3-6] It has been postulated that carcinogenic PAHs of this class may undergo metabolic activation by a novel pathway that involves formation of a bridge carbocation intermediate capable of directly alkylating DNA[7] in addition to the diol epoxide pathway established for the parent PAHs without a bridge.[8]



**1**



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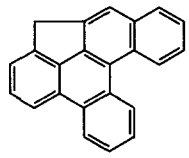

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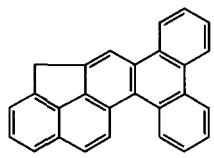

**4**

In connection with investigations of the mechanisms of carcinogenesis of bridged PAHs, we required an efficient method for the synthesis of **1** and its higher polycyclic analogs. Although several syntheses of **1** have been described,[9-12] none of these methods were entirely satisfactory because of the numbers of steps and the relatively low overall yields obtained. We now report an improved synthetic approach which provides **1** in 50% overall yield from available precursors in four steps and application of the method to the synthesis of the previously unknown polycyclic hydrocarbons 8*H*-benzo[*g*]cyclopenta[*mno*]chrysene (**3**) and 4*H*-benzo[*f*]cyclopenta[*pqr*]picene (**4**).