46, 103481-93-2; **47a**, 103461-17-2; **17b**, 103461-16-1; **48a**, 103461-15-0; **48b**, 103461-14-9; **49a**, 103461-07-0; **49b**, 103461-10-5; **50a**, 114028-11-4; **50b**, 114028-12-5; **51**, 103481-82-9; **52**, 103481-97-6; **53a**, 103461-05-8; **53b**, 103461-06-9; **54**, 114028-13-6; **55**, 114028-14-7; **56**, 114028-15-8; **57**, 114028-17-0; **58**, 103449-53-2; **59**, 103460-98-6; **60**, 103481-74-9; **61**, 103481-91-0; **62a**, 103460-96-4; **62b**, 103460-97-5; **63a**, 113925-86-3; **63b**, 103461-00-3; **64a**, 103449-66-7; **64b**, 103449-67-8; **65**, 103449-68-9; **66**, 103461-29-6; **67**, 103461-28-5; **68**, 103461-30-9; **69**, 103461-42-3; **70a**, 103461-38-7; **70b**, 103461-40-1; **71**, 113925-87-4.

Dinucleotide Analogues as Inhibitors of Thymidine Kinase, Thymidylate Kinase, and Ribonucleotide Reductase

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P^1-(Adenosine-5′)-P^n-(thymidine-5′) tri-, tetra-, penta-, and hexaphosphates (Ap_nT) plus the analogues with a methylene group α,β to the thymidine residue (Ap_ncpT) were synthesized by coupling the appropriate two nucleotides, having activated one by morpholine. These were tested as potential dinucleotide inhibitors of thymidine kinase, thymidylate kinase, and ribonucleotide reductase. All three enzymes bind ATP and thymidine or its nucleotides and therefore might be inhibited by dinucleotides containing adenosine and thymidine. Ap_5T and Ap_6T strongly inhibited all three enzymes (IC_{50} = 2.4–20 μM). Ap_4cpT and Ap_5cpT also strongly inhibited the two kinases (IC_{50} = 4–20 μM) but were much weaker inhibitors of the reductase (IC_{50} = 130 and 230 μM).

Thymidine kinase (EC 2.7.1.21), thymidylate kinase (EC 2.7.4.9), and ribonucleotide reductase (EC 1.17.4.1) are all elevated in malignancy and are essential to DNA synthesis. All three enzymes bind ATP and thymidine or its nucleotides and therefore might be inhibited by dinucleotides containing adenosine and thymidine. Bisubstrate and transition-state analogues have proved effective inhibitors of some enzymes (e.g. Ap_5A inhibits adenylate kinase[1] and PALA inhibits aspartate transcarbamylase[2]); also Hampton et al.[3] have found Ap_3T to be a moderate inhibitor of thymidine kinase. We have synthesized the series Ap_3T–Ap_6T and the corresponding analogues with a methylene group α,β to the thymidine residue and have tested them against the three enzymes of interest. It has been previously shown that insertion of the α,β-methylene residue can confer increased enzyme inhibitor potency;[4] this would also be expected to confer increased chemical and enzymatic stability.

Chemistry

We explored three ways of coupling the nucleoside phosphates: (a) use of 1,1′-carbonyldiimidazole,[5] (b) use of diphenyl chlorophosphate,[6] (c) condensation by the morpholidate method.[7] We found (c) to be the best, particularly for longer chain lengths. Yields of 20% or greater were obtained except in the case of Ap_6T and Ap_5cpT. For Ap_5T our yield (22%) was substantially greater than that of Bone et al.[8] (7.5%). We found that the carbonyldiimidazole route gave a good yield (29%) only in the synthesis of Ap_2cpT (from AMP and α,β-methylene-TDP). In the other reactions that we tried, namely TMP plus either ADP or ATP and Ap_4 plus α,β-methylene-TDP, we were unable to isolate pure product. The diphenyl chlorophosphate route gave good yields of coupling, but owing to disproportionation reactions the major product contained one less phosphorus atom than expected. For example, ADP + TDP gave 6% Ap_4T and 20% Ap_3T and ATP + TDP gave 6% AP_5T and 9% Ap_4T. All compounds were characterized by ^1H and ^{31}P NMR, and their purity was assessed by HPLC. Compounds Ap_3T–Ap_5T, and Ap_2cpT–Ap_4cpT gave satisfactory elemental analyses (CHNP). After material was reserved for biological testing, there was insufficient material for the elementary analysis of either Ap_6T or Ap_5cpT.

Table I

Inhibition of Thymidine Kinase: IC_{50},[a] μM			
Ap_3T	>500	Ap_2cpT	>500
Ap_4T	15.0 (±2.5)	Ap_3cpT	17.0 (±2.2)
Ap_5T	2.5 (±0.3)	Ap_4cpT	9.2 (±0.3)
Ap_6T	6.0 (±0.6)	Ap_5cpT	19.7 (±2.7)
Ap_5A	>500		
Inhibition of Thymidylate Kinase: IC_{50}, μM			
Ap_3T	1230 (±180)	Ap_2cpT	730 (±70)
Ap_4T	290 (±40)	Ap_3cpT	340 (±60)
Ap_5T	8 (±2)	Ap_4cpT	6 (±1)
Ap_6T	6 (±1)	Ap_5cpT	4 (±1)
Ap_5A	>1000		
Inhibition of Ribonucleotide Reductase: IC_{50}, μM			
Ap_3T	>500	Ap_2cpT	460
Ap_4T	77 (±4)	Ap_3cpT	300 (±10)
Ap_5T	14 (±3)	Ap_4cpT	130 (±10)
Ap_6T	20 (±6)	Ap_5cpT	230 (±10)
Ap_5A	>500		

[a] IC_{50} is the concentration of inhibitor required in an assay to reduce the control enzyme rate by 50%. Figures in parentheses are the standard errors.

Biological Results

Thymidine Kinase. By analogy with adenylate kinase, Ap_4T would be expected to be the best inhibitor. We found that Ap_4T was a better inhibitor than Ap_3T, but that unexpectedly the compounds with longer chain lengths were even more potent, with Ap_5T being the best inhibitor of the series. These results are broadly in agreement with those of Bone et al.,[8] but we found a greater difference in potency between the compounds. This may be due to the

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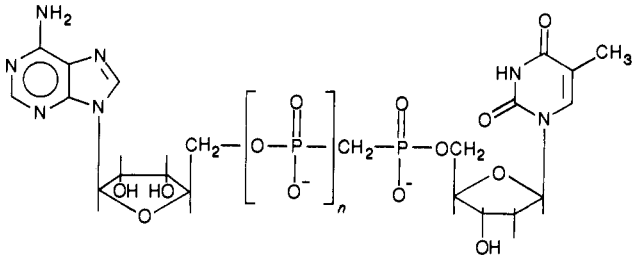
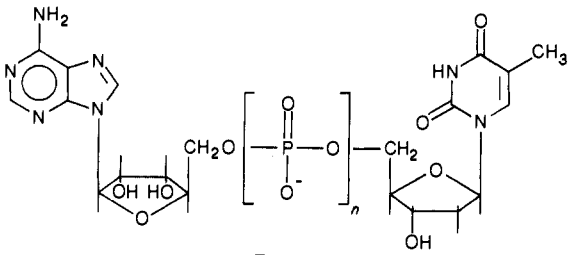


Ap_2cpT, $n = 2$
Ap_3cpT, $n = 3$
Ap_4cpT, $n = 4$
Ap_5cpT, $n = 5$



Ap_3T, $n = 3$
Ap_4T, $n = 4$
Ap_5T, $n = 5$
Ap_6T, $n = 6$

different enzyme sources used. The presence of an α,β-methylene group had no significant effect on the shorter chains but slightly reduced the activity of the compounds with five or six phosphorus atoms. Ap_5A was inactive, showing that the pyrimidine portion of the molecule was essential for binding.

Thymidylate Kinase. Again by analogy with adenylate kinase, Ap_5T would be expected to be the best inhibitor. We found (Table I), as did Bone et al.,[9] that both Ap_5T and Ap_6T strongly inhibited the enzyme, with Ap_6T being the best inhibitor. The presence of an α,β-methylene group seemed to make very little difference to the inhibition. As with thymidine kinase, Ap_5A did not inhibit the enzyme.

Ribonucleotide Reductase. Ap_5T and Ap_6T were potent inhibitors of CDP reduction (Table I). The series containing an α,β-methylene group was much less active. As with the other two enzymes, the pyrimidine portion is essential as Ap_5A did not inhibit.

Conclusion

The optimal chain length for inhibition of these three enzymes is either five or six phosphorus atoms, there being only small differences in the inhibition caused by compounds with these two chain lengths. However, for thymidylate kinase the limit may not yet have been reached and a chain length of seven phosphorus atoms may be better still. All three enzymes could tolerate the presence of a methylene group α,β to the thymidine, although with considerable loss of binding affinity in the case of ribonucleotide reductase. Replacement of the central oxygen atom in pyrophosphate with a methylene group (OPOPO vs OPCPO) will decrease the angle about the central atom by 11.7°.[10] As a consequence of this and the longer P–C bond, there is a 16% increase in the distance between the terminal oxygens.[11] Either this increase, or the loss of binding to the polar oxygen atom may be the reason for the weaker inhibition of ribonucleotide reductase by the α,β-methylene compounds. Any effect of the methylene group on pK_a values is not relevant as all the compounds will be fully ionized at physiological pH. Ribonucleotide reductase is the only one of these three enzymes where the binding of ATP does not result in the direct transfer of a phosphoryl group. ATP is a positive effector that binds to the M1 subunit of the enzyme and is an essential requirement for CDP reduction. TTP is a negative effector of CDP reduction with an IC_{50} value of 19.3 μM (SE ±0.15) for the L1210 enzyme preparation described here. It is possible that Ap_5T and Ap_6T may have the structural requirements to span the ATP and TTP binding sites on the M1 subunit of ribonucleotide reductase and thus represent a new class of inhibitor.

In all cases, Ap_5A was inactive, showing that the pyrimidine portion of the molecule was essential for binding.

Experimental Section

Chemical Synthesis. ^1H NMR spectra were determined with a Bruker WM 250 spectrometer with sodium 3-(trimethylsilyl)-propanesulfonate as the internal standard. ^{31}P NMR were determined with a Bruker HFX90 spectrometer with 85% phosphoric acid as the external standard. Where indicated, ethylenediaminetetraacetic acid was added to improve resolution. Analytical HPLC was performed with a 10 cm × 4.6 mm stainless steel column packed with Spherisorb S5 NH_2 and eluted with 0.3 M ammonium phosphate of the appropriate pH (4–6) for each sample. Detection was at 254 and 280 nm, and quantitation was carried out with a Trilab (Trivector, Sandy, Beds). Preparative LC was carried out on Merck Kieselgel 60 (Art. 15111) with a Jobin-Yvon Chromatospac Prep 10 with UV monitoring at 254 nm. Thin-layer chromatograms were run on fluorescent silica (Merck 5735) with spot location by UV light, the cysteine/sulfuric acid reagent[12] for 2′-deoxynucleosides, and the tetramethylbenzidine periodate reagent[13] for ribonucleosides. Ion-exchange columns contained A25 DEAE-Sephadex and were eluted with linear gradients from a Pharmacia P3 peristaltic pump.

Sodium, pyridinium, and morpholinium salts of the nucleotides were prepared with use of AG50WX4 ion-exchange resins; tributylammonium and trioctylammonium salts were prepared by the addition of an ethanolic solution of the free base to the pyridinium salt of the nucleotide and subsequent concentration. Nucleotides were dried before use by repeated evaporations with the appropriate dry solvent. The final products were obtained as their sodium salts by precipitation from water with AR ethanol followed by centrifugation and drying. Dimethylacetamide (DMA) was dried by storage over activated 4A molecular sieves.

P^1-(Adenosine-5′)-P^4-(thymidine-5′) Tetraphosphate (Ap_4T). Diphenyl chlorophosphate (1.0 mmol) and tri-*n*-butylamine (1.0 mmol) were added to a solution of the tris(tri-*n*-octylammonium salt) of ATP (0.75 mmol) in 6 mL of dioxane/DMA (2:1). The mixture was stirred for 4 h, the solvent was evaporated, and then anhydrous ether (30 mL) was added. The flask was shaken until all oily material had disappeared and then was kept in the refrigerator for 1 h. The ether was pipetted off, leaving a white solid; residual ether was removed by evaporation with dioxane. More dioxane (2 mL) was added, followed by a solution of the bis(tri-*n*-octylammonium salt) of TDP (1.1 mmol) in pyridine (2 mL). The solution was stirred and occasionally shaken for 1 week. Solvent was then removed by evaporation and ether added, giving a white solid. The latter was filtered off and dissolved in water (50 mL) and 3 N aqueous NH_3 was added to neutrality. After several extractions with ether, the solution was concentrated (20 mL) and applied to a DEAE-Sephadex column (HCO_3^-) (1.6 × 25 cm), which was eluted from 0.3–0.8 M triethylammonium bicarbonate (pH 7.8). The partially purified product was collected and applied to a silica column (200 g), which was eluted with *n*-PrOH/2.5 N aqueous NH_3 (3:1). The product was precipitated as its sodium salt to give 70 mg (9%) of a white

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solid (96% pure on HPLC). ^{1}H NMR (D_2O): δ 1.83 (3 H, s, 5-CH_3), 2.26 (2 H, m, 2'-CH_2), 4.1–4.3 (5 H, m), 4.39 (1 H, s), 4.6 (2 H, m), 4.65 (s), 6.09 (1 H, d, 1'-CH (A)), 6.20 (1 H, t, 1'-CH (T)), 7.59 (1 H, s, 6-CH), 8.20 (1 H, s, ring proton (A)), 8.51 (1 H, s, ring proton (A)). ^{31}P NMR (D_2O): δ −11.6 (2 P, m, CH_2OP), −23.2 (2 P, m, POP). Anal. ($C_{20}H_{25}N_7O_{20}P_4Na_4 \cdot 3H_2O \cdot 0.5EtOH$) C, H, N, P.

P^1-(Adenosine-5')-P^5-(thymidine-5') Pentaphosphate (Ap_5T). A solution of the bis(tri-*n*-octylammonium salt) of TDP (1.4 mmol) in dry DMA (10 mL) was added to ATP morpholidate[7] (0.47 mmol). A clear solution was obtained within 30 min. After 3 days the solvent was evaporated and the residue partitioned between water and ether. The aqueous solution was applied to a DEAE-Sephadex column (HCO_3^-) (2.6 × 28 cm). The column was eluted from 0.3–0.6 M triethylammonium bicarbonate (pH 7.8). The partially purified product was collected and applied to a silica column (205 g), which was eluted with *n*-PrOH/2 N aqueous NH_3 (7:3). The product was precipitated as its sodium salt, giving 0.11 g (22%) of white solid, which was 98% pure on HPLC. ^{1}H NMR (D_2O): δ 1.87 (3 H, s, 5-CH_3), 2.30 (2 H, m, 2'-CH_2), 4.1–4.7 (m, sugar CH's), 6.12 (1 H, d, 1'-CH (A)), 6.25 (1 H, t, 1'-CH (T)), 7.64 (1 H, s, 6-CH), 8.20 (1 H, s, ring H (A)), 8.50 (1 H, s, ring H (A)). ^{31}P NMR (D_2O + EDTA): δ −11.5 (2 P, q, CH_2OP), −22.8 (3 H, t, POPOP). Anal. ($C_{20}H_{25}N_7O_{23}P_5 1.5H_2O \cdot EtOH$) C, H, N, P.

P^1-(Adenosine-5')-P^6-(thymidine-5') Hexaphosphate (Ap_6T). A solution of the tris(tri-*n*-octylammonium salt) of TTP (0.83 mmol) in dry DMA (8 mL) was added to ATP morpholidate[7] (0.42 mmol). The solution soon became clear. Three days later the solvent was evaporated and the residue partitioned between ether and dilute ammonia. The aqueous layer was concentrated, and the residue was dissolved in water and applied to a DEAE-Sephadex column (HCO_3^-) (2.6 × 28 cm). The column was eluted from 0.35–0.75 M triethylammonium bicarbonate (pH 7.7). The partially purified product was collected and applied to a silica column (210 g), which was eluted with *n*-PrOH/2.5 N aqueous NH_3 (3:1). The product was precipitated as its sodium salt, giving 21 mg (4%) of white solid (98% on HPLC). ^{1}H NMR (D_2O): δ 1.88 (3 H, s, 5-CH_3), 2.31 (2 H, t, 2'-CH_2), 4.20 (3 H, m), 4.29 (2 H, br s), 4.41 (1 H, s), 4.6 (1 H, m), 4.65 (1 H, s), 4.8 (1 H, m), 6.10 (1 H, d, 1'-CH (A)), 6.27 (1 H, t, 1-CH (T)), 7.69 (1 H, s, 6-CH), 8.34 (1 H, s, ring proton (A)), 8.58 (1 H, s, ring proton (A)). ^{31}P NMR (D_2O): δ −11.7 (2 P, s, CH_2OP), −22.9 (4 P, s, POP).

α,β-Methylene TDP Morpholidate. A solution of dicyclohexylcarbodiimide (DCC, 7.0 mmol) in *tert*-butyl alcohol (7 mL) was added to a refluxing solution of the morpholinium salt of α,β-methylene-TDP[14] (0.70 mmol) and morpholine (4.9 mmol) in 50% aqueous *tert*-butyl alcohol (14 mL). The solution was refluxed for 3 h and then allowed to cool overnight. The mixture was filtered, concentrated (5 mL), and partitioned between water and ether. The aqueous layer was concentrated to dryness and then evaporated with ethanol and then toluene to yield 0.91 g of a pale yellow solid, which still contained solvent. This slightly impure dicarboxamidine salt was used without further purification.

P^1-(Thymidine-5')-P^3-(adenosine-5') (P^1,P^2-Methylene)-triphosphate (Ap_2cpT). A solution of the tributylammonium salt of AMP (0.10 mmol) in dry DMA (1 mL) was added to a stirred solution of α,β-methylene-TDP morpholidate (ca. 0.05 mmol) in dry DMA (1 mL). The next day the solvent was evaporated and the product applied to a silica column (130 g), which was eluted with *n*-PrOH/3 N aqueous NH_3 (3:1). The product was precipitated as its sodium salt, giving 18 mg (40%) of a white solid, which was pure on HPLC (>99%). ^{1}H NMR (D_2O): δ 1.81 (3 H, s, 5-CH_3), 2.25 (2 H, m, 2'-CH_2), 2.4 (2 H, m, PCH_2P), 4.09 (3 H, s), 4.25 (2 H, s), 4.38 (1 H, s), 4.54 (2 H, m), 4.7 (1 H, m), 6.08 (1 H, d, 1'-CH (A)), 6.19 (1 H, t, 1'-CH (T)), 7.56 (1 H, s, 6-CH), 8.18 (1 H, s, ring H (A)), 8.5 (1 H, br s, ring H (A)). ^{31}P NMR (D_2O + EDTA): δ 16.6 (1 P, d, TOP), 7.2 (1 P, dd, CPO), −11.2 (1 P, d, AOP). Anal. ($C_{21}H_{27}N_7O_{16}P_3Na_3 \cdot 6H_2O \cdot 0.5EtOH$) C, H, N, P.

P^1-(Thymidine-5')-P^4-(adenosine-5') (P^1,P^2-Methylene)-tetraphosphate (Ap_3cpT). A solution of the mono(tri-*n*-octylammonium salt) of ADP (0.15 mmol) in dry DMA (1 mL) was added to a stirred solution of α,β-methylene-TDP morpholidate (ca. 0.20 mmol) in dry DMA (1 mL). Eight days later the solvent was evaporated and the product eluted from a silica column (200 g) with *n*-PrOH/3 N aqueous NH_3 (7:3). The product was precipitated as its sodium salt, giving 28 mg (20%) of a white powder (95% pure on HPLC). ^{1}H NMR (D_2O): δ 1.84 (3 H, s, 5-CH_3), 2.28 (2 H, t, 2'-CH_2), 2.40 (2 H, t, PCH_2P), 4.08 (3 H, s), 4.25 (2 H, m), 4.39 (1 H, m), 4.58 (2 H, m), 4.63 (1 H, m), 6.10 (1 H, d, 1'-CH (A)), 6.22 (1 H, t, 1'-CH (T)), 7.60 (1 H, s, 6-CH), 8.20 (1 H, s, ring H (A)), 8.5 (1 H, br s, ring H (A)). ^{31}P NMR (D_2O): δ 16.5 (1 P, d, TOP), 7.4 (1 P, dd, CPO), −11.7 (1 P, d, AOP), −23.0 (1 P, t, POP). Anal. ($C_{21}H_{27}N_7O_{19}P_4Na_4 \cdot 9H_2O$) C, H, N, P.

P^1-(Thymidine-5')-P^5-(adenosine-5') (P^1,P^2-Methylene)-pentaphosphate (Ap_4cpT). A solution of the bis(tri-*n*-octylammonium salt) of α,β-methylene-TDP (0.40 mmol) in dry DMA (1.5 mL) was added to the carboxamide salt of ATP morpholidate[7] (0.25 mmol). The flask was gently warmed to dissolve all the solids and then the contents were stirred at room temperature. Nine days later the solvent was evaporated and the residue partitioned between ether (10 mL) and 2 N aqueous NH_3 (10 mL). The aqueous layer was concentrated, and the residue was redissolved in water and filtered before application to a DEAE-Sephadex column (HCO_3^-) (1.6 × 25 cm). The latter was eluted from 0.3–0.7 M triethylammonium bicarbonate. The product was precipitated as its sodium salt to give 92 mg (32%) of a white solid (95% pure on HPLC). ^{1}H NMR (D_2O): δ 1.86 (3 H, s, 5-CH_3), 2.30 (2 H, t, 2'-CH_2), 2.43 (2 H, t, PCH_2P), 4.1 (3 H, m), 4.25 (2 H, m), 4.40 (1 H, m), 4.6 (2 H, m), 4.66 (1 H, s), 6.11 (1 H, d, 1'-CH (A)), 6.26 (1 H, t, 1'-CH (T)), 7.65 (1 H, s, 6-CH), 8.22 (1 H, s, ring H (A)), 8.54 (1 H, s, ring H (A)). ^{31}P NMR (D_2O): δ 16.7 (1 P, d, TOP), 7.7 (1 P, m, CPO), −11.6 (1 P, d, AOP), −23 (2 P, m, POP). Anal. ($C_{21}H_{27}N_7O_{22}P_5Na_5 \cdot 6H_2O \cdot EtOH$) C, H, N, P.

Adenosine Tetraphosphate Morpholidate. A solution of DCC (3.5 mmol) and morpholine (2.3 mmol) in *tert*-butyl alcohol (15 mL) was added dropwise over 3 h to a refluxing solution of the morpholinium salt of adenosine tetraphosphate (0.57 mmol) in 50% aqueous *tert*-butyl alcohol (25 mL). Reflux was continued for 4 h. The next day the solution was filtered, evaporated, and partitioned between water (20 mL) and ether (2 × 30 mL). The aqueous layer was concentrated (3 mL), filtered, and applied to a DEAE-Sephadex (HCO_3^-) column (25 × 1.6 cm), which was eluted from 0.3–0.8 M triethylammonium bicarbonate. The product-containing fractions were evaporated to dryness and then concentrated with methanol (4 × 30 mL). Methanol (20 mL) and *N*,*N*'-dicyclohexyl-4-morpholinecarboxamidine (0.70 g) were added. The product was precipitated from methanol (4 mL) with ether to yield 0.33 g (30%) of product, which was used without further purification.

P^1-(Thymidine-5')-P^6-(adenosine-5') (P^1,P^2-Methylene)-hexaphosphate (Ap_5cpT). This was made and purified in the same way as Ap_4cpT starting from α,β-methylene-TDP[14] (0.25 mmol) and adenosine tetraphosphate morpholidate (ca. 0.16 mmol). The precipitated sodium salt weighed 13 mg (4%) (95% pure on HPLC). ^{1}H NMR (D_2O): δ 1.87 (3 H, s, 5-CH_3), 2.32 (2 H, t, 2'-CH_2), 2.43 (2 H, t, PCH_2P), 4.14 (3 H, m), 4.20 (1 H, m), 4.29 (1 H, m), 4.41 (1 H, m), 4.65 (2 H, m), 4.8 (1 H, m), 6.11 (1 H, d, 1'-CH (A)), 6.27 (1 H, t, 1'-CH (T)), 7.68 (1 H, s, 6-CH), 8.23 (1 H, s, ring H (A)), 8.56 (1 H, s, ring H (A)). ^{31}P NMR (D_2O + EDTA): δ 16.7 (1 P, d, TOP), 7.8 (1 P, m, CPO), −11.6 (1 P, d, AOP), −22.6 (3 P, m, POPOP).

Assay Procedure. The concentration of each inhibitor was checked by measuring the UV absorption of the solutions at 260 nm (ε 23 000).

1. **Thymidine Kinase.** The cytoplasmic isoenzyme was isolated from L1210 cells with an affinity column[4] and assayed in the presence of 1 mM ATP, 1 mM $MgCl_2$, 10 μM ^{3}HTdR (150 μCi/μmol), 5% sucrose, 40 mM Tris, pH 7.8, with variable concentrations of inhibitor (0–500 μM). The data were fitted to a Dixon plot ($1/v$ vs I) by linear regression and IC_{50} values were

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then calculated (intercept/slope).

2. **Thymidylate Kinase.** Enzyme activity was measured essentially as described by Lee and Cheng[15] using [^{3}H]TMP (24 μM) and ATP (1.5 mM) as cosubstrates. A dialyzed extract of blast cells received from a patient with acute myeloid leukemia was used as the enzyme source. The data were fitted by a nonlinear least-squares regression program based on the algorithm described by Jennrich and Sampson.[16] For determination of IC_{50} values, the data were fitted to the equation (response) = $a + b$ ln [inhibitor] with an error weighting of $1/(y + y)^2$. [17,18]

3. **Ribonucleotide Reductase.** A crude extract was prepared from L1210 cells and adjusted to pH 5.2 with 1 M acetic acid. The precipitate was collected, dissolved in 50 mM Tris·HCl, pH 8.0, and used as the enzyme source. CDP reductase activity was measured as described by Cory and Mansell.[19] The data were analyzed in the same way as those for thymidylate kinase.

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Synthesis of the Tumorigenic 3,4-Dihydrodiol Metabolites of Dibenz[*a*,*j*]anthracene and 7,14-Dimethyldibenz[*a*,*j*]anthracene

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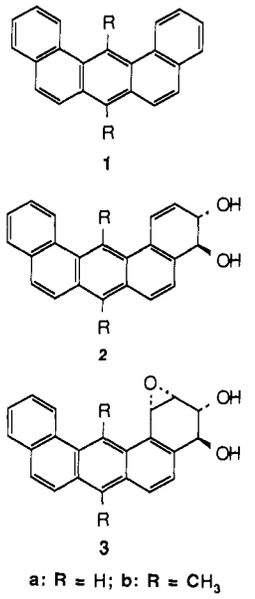
Syntheses are described of the *trans*-3,4-dihydrodiol derivatives (**2a** and **2b**) of dibenz[*a*,*j*]anthracene and 7,14-dimethyldibenz[*a*,*j*]anthracene (**1a** and **1b**), implicated as their *proximate* carcinogenic metabolites. Conversion of **2a** to the bay region *anti*-diol epoxide derivative **3a**, its putative *ultimate* carcinogenic metabolite, is also reported. The related diol epoxide derivative of **2b** could not be prepared due to its chemical instability. Tumorigenicity assays confirm that **1b** and **2b** are potent carcinogens on mouse skin, while **1a** and **2a** are only relatively weakly active. The diol epoxide **3a** exhibited significantly higher tumorigenicity than its dihydrodiol precursor **2a**. These findings are consistent with the hypothesis that the bay region diol epoxide metabolites are the active carcinogenic forms of these hydrocarbons. They also support the generalization that methyl substitution in bay regions enhances the carcinogenic activity of polycyclic aromatic hydrocarbons.

Introduction of methyl groups into the nonbenzo bay region positions of polycyclic aromatic hydrocarbons often markedly enhances their potency as carcinogens.[1,2] One of the most dramatic examples of this "bay region methyl effect" is 7,14-dimethyldibenz[*a*,*j*]anthracene (**1b**).[3] While the parent hydrocarbon dibenz[*a*,*j*]anthracene (**1a**) exhibits only weak borderline activity as a carcinogen on mouse skin, the dimethyl analogue is a potent carcinogen, its activity rivalling that of the highly potent 7,12-dimethylbenz[*a*]anthracene.[2]

It has been established that polycyclic hydrocarbons require metabolic activation to express their biological potential, and the principal active metabolites have been identified as the bay region diol epoxides.[4] As part of a program to elucidate the molecular basis of the "bay region methyl effect", we required the bay region *anti*-diol epoxides of **1a** and **1b** (**3a** and **3b**) and their 3,4-dihydrodiol precursors (**2a** and **2b**). We now report the synthesis of *trans*-3,4-dihydroxy-3,4-dihydrodibenz[*a*,*j*]anthracene (**2a**) *trans*-3,4-dihydroxy-3,4-dihydro-7,14-dimethyldibenz[*a*,*j*]anthracene (**2b**), and *trans*-3,4-dihydroxy-*anti*-1,2-epoxy-1,2,3,4-tetrahydrodibenz[*a*,*j*]anthracene (**3a**).



Results and Discussion

The synthetic approach to the dihydrodiols **2a** and **2b** is based upon the general method reported earlier for the conversion of phenols to dihydrodiols.[5,6] 3-Hydroxydibenz[*a*,*j*]anthracene (**9b**) required as the starting compound for the synthesis of **2a** was itself synthesized via the sequence in Scheme I. Metalation of the appropriate *N*,*N*-diethylarylamide with *sec*-butyllithium in ether by the method of Beak[7] furnished *N*,*N*-diethyl-2-lithio-6-methoxy-1-naphthamide (**5a**). Condensation of **5a** with

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