Efforts that have been made to stabilize PTMSP/I by UV treatment (254 nm, 7 min) have led to a significant reduction in permselectivity and permeation rates (Table I). Apparently, the integrity of the LB suprastructure cannot be fully maintained under these conditions; i.e., defects are created as a consequence of the two-dimensional polymerization and/or the photodecomposition of the support.

Our demonstration that molecular sieving can be achieved with LB composites, made from a combination of porous surfactants and highly permeable supports possessing a continuous surface, should lead the way to novel and potentially exploitable membranes for molecular separations. Efforts aimed at exploring such possibilities are now under intensive investigation.

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## Nucleotides Bearing a Cleavable Genotoxic Group on the Phosphate

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*N*-Nitrosopyrrolidine (NPYR), a rodent carcinogen,[1] is a promutagen requiring enzymic conversion, presumably to its α-hydroxylated derivative, to exhibit its mutagenic activity.[2] Earlier studies from our laboratory have shown that NPYR and *N*-nitrosomorpholine can be converted into their α-phosphate esters on near-ultraviolet (UVA) irradiation in the presence of inorganic phosphate and that these α-phosphate derivatives are directly mutagenic toward bacteria.[3-7] Furthermore, direct mutagenicity was observed for UVA-irradiated mixtures of *N*-nitrosomorpholine and nucleotides (in place of inorganic phosphate), and the mutagenic components formed were found in distinctive zones in paper chromatography, depending on the nucleotide used.[3] It is likely, therefore, that in this process the nucleotides were linked to the *N*-nitrosodialkylamine at the α-carbon. Such compounds seemed to us to be worthy of exploration for their properties. We wish to report here the synthesis of this new class of nucleotide derivatives using NPYR. These nucleotides are directly mutagenic to *Salmonella*, and they can be cleaved in vitro under mild conditions at the phosphoester–NPYR linkage. With near-ultraviolet irradiation, this cleavage takes place and, when a strand of DNA is present in the reaction mixture, the DNA undergoes single strand breaks.

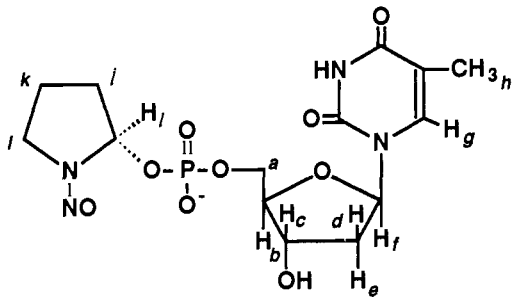

Figure 1. Structure of NPYR-dpT.

Table I. Preparation and Properties of NPYR Nucleotides

| compd[a] | yield,[b] % | PPC[c] $R_f$ | PPC[c] $R_f$ of parent nucleotide | PEP[d] $M_{pX}$ | UV in $H_2O$[e] $\lambda_{max}$, nm |
|---|---|---|---|---|---|
| NPYR-dpT | 9 | 0.70 | 0.37 | 0.54 | 266 |
| NPYR-dpC | 15 | 0.62 | 0.21 | 0.51 | 272 |
| NPYR-dpA | 11 | 0.62 | 0.27 | 0.52 | 259 |
| NPYR-dpG | 12 | 0.44 | 0.12 | 0.51 | 248 |
| NPYR-dpCpT | 8 | 0.33 | 0.19 | 0.74 | 268 |
| NPYR-pA | 14 | 0.62 | 0.26 | 0.47 | 258 |
| NPYR-Up | 4 | 0.53 | 0.22 | 0.51 | 259 |

[a] dpX represents deoxyribonucleoside 5′-phosphate, pA is adenosine 5′-phosphate, and Up is uridine 2′(and 3′)-phosphate. [b] The yields were from α-acetoxy-NPYR used and are calculated on the basis of UV absorbance of the isolated material. Molar absorbances at the $\lambda_{max}$ are the calculated sums of those for nucleotides and α-acetoxy-NPYR. The yield for NPYR-dpCpT was from dpCpT, for which α-acetoxy-NPYR was used in excess. [c] Paper chromatography was run ascendingly on Toyo filter paper 51C, with propanol–concentrated $NH_4OH$–$H_2O$ (6:3:1, v/v) as solvent. [d] Paper electrophoresis was run in 0.03 M sodium phosphate buffer at pH 7.4 (200 V, 80 min). $M_{pX}$ represents mobility relative to that of the parent nucleotide. [e] A feature in the spectra of these NPYR nucleotides was that the $A_{240}$ values are elevated from those of their parent nucleotides due to the intramolecular presence of an equimolar NPYR moiety.

A solution of thymidine 5′-phosphate $Na_2$ (dpT) (8 mg) and α-acetoxy-NPYR (2 mg)[8] in water (16 μL) was heated at 75 °C for 20 min. The product, thymidine 5′-phosphate mono(1-nitroso-2-pyrrolidinyl) ester (NPYR-dpT, Figure 1), was isolated by use of TLC on cellulose [solvent: 2-propanol–concentrated $NH_4OH$–$H_2O$ (7:1:2)] followed by paper chromatography [solvent: propanol–concentrated $NH_4OH$–$H_2O$ (6:3:1)]. The NMR spectra ($^1H$, $^{31}P$, $^1H$–$^1H$ COSY, and $^1H$–$^{31}P$ HSQC) of this material supported the expected structure and its diastereomeric mixture: $^1H$ NMR (500 MHz, $D_2O$) δ 1.87 (s, h), 1.92 (s, h), 2.09–2.28 (m, j + k), 2.28–2.41 (m, d + e), 3.48–3.74 (m, l), 3.97–4.19 (m, a + b), 4.51–4.58 (m, c), 6.30–6.38 (m, f), 6.53–6.58 (m, i), 7.70 (s, g), and 7.72 (s, g); $^{31}P$ NMR (202.35 Hz, $D_2O$) δ −2.44 and −2.83. The proton-detected $^1H$–$^{31}P$ heteronuclear two-dimensional correlation spectrum ($^1H$–$^{31}P$ HSQC) confirmed the assignment that the phosphate is linked to the α-carbon of NPYR: both H(i) and H(a + b) were correlated with the $^{31}P$.

The mass spectrum gave signals corresponding to the assigned structure: *m/z* 421 (free acid), 438 ($NH_4$ salt), and 443 (Na salt). The UV spectrum in water was close to the sum of the spectra for dpT and α-acetoxy-NPYR [the ε values of which are 6850 at 229 nm ($\lambda_{max}$), 5250 at 240 nm, and less than 100 at 280 nm and longer wavelengths]; the spectrum at pH 12, which was stable, showed a maximum at 265 nm with a 20% lower absorbance from that in water, as expected for a thymine nucleotide. At pH 2, the spectrum showed a rapid change, as monitored by the decrease in absorbance at 240 nm, to give, after 5 min, a spectrum identical to that of dpT, a phenomenon suggesting the cleavage of the NPYR moiety. On treatment with snake venom phosphodiesterase, NPYR-dpT gave dpT, as identified by paper chroma-

(1) Druckrey, H.; Preussmann, R. *Naturwissenschaften* **1962**, *49*, 489–499.
(2) McCann, J.; Choi, E.; Yamasaki, E.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 5135–5139.
(3) Hayatsu, H.; Shimada, H.; Arimoto, S. *Gann (Jpn. J. Cancer Res.)* **1984**, *75*, 203–206.
(4) Shimada, H.; Hayatsu, H. *Mutation Res.* **1985**, *143*, 165–168.
(5) Shimada, H.; Yakushi, K.; Ikarashi, A.; Mochizuki, M.; Suzuki, E.; Okada, M.; Yokoyama, S.; Miyazawa, T.; Hayatsu, H. *Relevance of N-Nitroso Compounds to Human Cancer: Exposures and Mechanisms*; Bartsch, H., et al., Eds.; IARC Scientific Publications No. 84; IARC: Lyon, 1987; pp 364–366.
(6) Arimoto, S.; Shimada, H.; Ukawa, S.; Mochizuki, M.; Hayatsu, H. *Biochem. Biophys. Res. Commun.* **1989**, *162*, 1140–1146.
(7) Mochizuki, M.; Anjo, T.; Sekiguchi, N.; Ikarashi, A.; Suzuki, A.; Wakabayashi, Y.; Okada, M. *Chem. Pharm. Bull.* **1986**, *34*, 3956–3959.
(8) Saavedra, J. E. *Tetrahedron Lett.* **1978**, *22*, 1923–1926.

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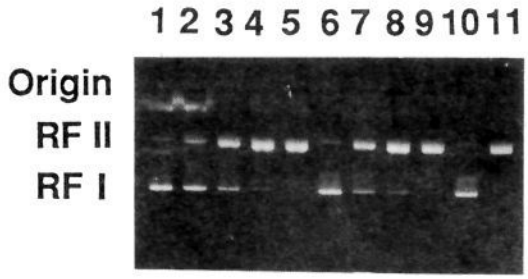


**Figure 2.** DNA single strand breaks induced by NPYR-dpT (and NPYR-pA) and UVA. Detection of DNA strand breaks was carried out with phage M13mp2 RF DNA using agarose gel electrophoresis as reported previously.[17] A change from form RFI to RFII is the indication for single strand breaks. The double-stranded covalently closed circular DNA (RFI) was prepared according to the literature.[18] DNA (10 ng/μL) in 20 mM sodium phosphate buffer (pH 7.4) was incubated with the reagents in a 96-well microtiter plate at 24 °C under UVA irradiation at 6.7 μW/mm$^2$. The volume of the reaction mixture was 40 μL. After a 3-h irradiation, aliquots (9 μL) were electrophoresed in 1% agarose gel with the running buffer, 89 mM Tris–89 mM boric acid–2.5 mM disodium EDTA, pH 8.3. Lane 1, no addition (DNA only); lane 2, UVA only; lanes 3–5, UVA + NPYR-pA, 0.05 mM, 0.25 mM, 0.75 mM, respectively; lane 6, NPYR-pA 0.75 mM only; lanes 7–9, UVA + NPYR-dpT, 0.05 mM, 0.25 mM, 0.75 mM, respectively; lane 10, NPYR-dpT 0.75 mM only; lane 11, a positive control: NPYR (1 mM) + UVA.[5]

tography and paper electrophoresis. After the acid or phosphodiesterase treatment, the solution became positive in the Bandrowski aldehyde test.[9] It has been suggested that 2-butenal is formed upon degradation of α-acetoxy-NPYR.[10] We found that NPYR-dpT (in an 8 mM aqueous solution at pH 6) can also be decomposed by UVA irradiation (320–400 nm, 6 μW/mm$^2$, 3 h, without appreciable changes in the pH) to give dpT.

We have prepared the NPYR phosphoesters from other deoxyribonucleoside 5′-phosphates and ribonucleoside phosphates. A dideoxynucleotide, dpCpT, was also derivatized. The preparation and properties of these derivatives are summarized in Table I.[11] All of these NPYR nucleotides are direct-acting mutagens, showing activities toward *Salmonella typhimurium* TA1535, a tester strain for base change mutations.[12] Their mutagenic potencies were similar to that of α-acetoxy-NPYR.[13]

DNA single strand breaks were caused by NPYR-pA and NPYR-dpT on UVA irradiation. Either the NPYR nucleotide alone or UVA alone was without the effect (Figure 2). The reaction seems to be mediated by active oxygen radicals, possibly •OH, because the breaks were inhibited by formate, thiourea, and cysteamine, which are scavengers of hydroxyl radical;[14] superoxide dismutase did not inhibit the strand break (data given in the supplementary material).

The NPYR moiety may be incorporated into oligonucleotides having terminal phosphomonoester groups. Such oligonucleotides would be useful in specific cleavage of nucleic acids[15] and in targeted gene-manipulation. This new class of nucleotide derivatives may also be useful in studies of mutagenesis and carcinogenesis mechanisms of *N*-nitrosodialkylamines. 2-Butenal, an α,β-unsaturated aldehyde generated on cleavage of the NPYR moiety from the NPYR nucleotides, should be reactive not only to DNA but also to nucleophilic groups in proteins and other biological substances.[16]

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**Supplementary Material Available:** Figure showing the gel electrophoresis of antioxidant inhibition of DNA single strand breaks induced by NPYR-dpT and UVA (2 pages). Ordering information is given on any current masthead page.

(9) Feigl, F. *Spot Tests in Organic Analysis*; Elsevier–Maruzen: Tokyo, 1960; pp 228–229.
(10) Chung, F.-L.; Wang, M.; Hecht, S. S. *Cancer Res.* **1989**, *49*, 2034–2041.
(11) With dpCpT, the esterification took place for the terminal 5′-phosphate but not for the internucleotide linkage. All of these NPYR esters decomposed slowly at neutral pH, but rapidly in acid (at pH 2, 5 min at room temperature; at pH 4 and 37 °C, 1 h) to give the parent nucleotides.
(12) Ames, B. N.; McCann, J.; Yamasaki, E. *Mutation Res.* **1975**, *31*, 347–364.
(13) The mutagenic activities of the NPYR nucleotides, as assayed by the plate incorporation method,[12] ranged from 160 to 250 His$^+$ revertants/plate/0.01 μmol; that of α-acetoxy-NPYR was 520 revertants/plate/0.01 μmol, with a solvent control value of 6–15 revertants/plate.
(14) Halliwell, B.; Gutteridge, J. M. C. *Free Radicals in Biology and Medicine*; Clarendon Press: Oxford, 1985; pp 25–45.
(15) Dervan, P. B. *Nature* **1992**, *359*, 87–88.
(16) Carey, F. A.; Sundberg, R. J. *Advanced Organic Chemistry, Part B: Reactions and Synthesis*, 3rd ed.; Plenum Press: New York, 1990; pp 39–46.
(17) Wakata, A.; Oka, N.; Hiramoto, K.; Yoshioka, A.; Negishi, K.; Wataya, Y.; Hayatsu, H. *Cancer Res.* **1985**, *45*, 5867–5871.
(18) Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: New York, 1989; pp 4.31–4.32.

## Preparation and Characterization of Singly Oxidized Metalloporphyrin Dimers: $[M(OEP^{\bullet/2})]_2SbCl_6$, M = Cu, Ni. Photosynthetic Special Pair Models

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We report the preparation and physical characterization (X-ray, UV–visible–near IR, IR, EPR, magnetic susceptibility) of two new metalloporphyrin π-cation radical derivatives formally analogous to the radical cation of the photosynthetic reaction center special pair.[2] The complexes are $[M(OEP^{\bullet/2})]_2^+$ (M = Ni or Cu),[3] and they possess a single electron hole per *pair* of porphyrin rings; both form discrete dimers in the solid state. The absence of an obvious covalent bond in these dimers distinguishes them from all previously characterized "partially oxidized" bis(porphyrin) systems.

Oxidation of [M(OEP)] in $CH_2Cl_2$ with 0.5 equiv of $[(4\text{-}BrPh)_3N]SbCl_6$ yields $[M(OEP^{\bullet/2})]_2SbCl_6$, which shows the empirical, porphyrin ring oxidation IR marker band.[4,5] The nickel

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(2) (a) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *Nature (London)* **1985**, *318*, 618. (b) Kirmaier, C.; Holten, D. *Photosynth. Res.* **1987**, *13*, 225. (c) Budil, D.; Gast, P.; Chang, C. H.; Schifer, M.; Norris, J. R. *Annu. Rev. Phys. Chem.* **1987**, *38*, 561.
(3) Abbreviations: Ct, center of porphyrin ring; $N_p$, porphinato nitrogen; RC, photosynthetic reaction centers; OEP, TPP, TnPrP, and P, dianions of octaethyl-, tetraphenyl- tetra-*n*-propylporphyrin, and a generalized porphyrin, respectively; π-cation radical derivatives are indicated with a raised dot in the formula (i.e., $OEP^{\bullet}$).
(4) Shimomura, E. T.; Phillippi, M. A.; Goff, H. M.; Scholz, W. F.; Reed, C. A. *J. Am. Chem. Soc.* **1981**, *103*, 6778.

0002-7863/93/1515-1181$04.00/0 