1-Oxidopyridin-2-yldiazomethane: a Water-soluble Alkylating Agent for Nucleosides and Nucleotides

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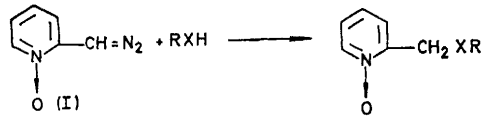
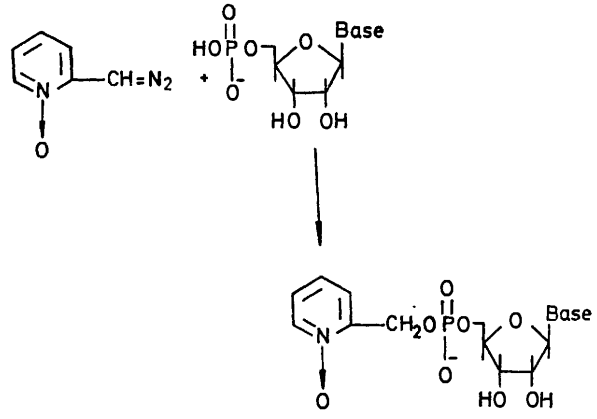
Summary The water-soluble alkylating agent: 1-oxidopyridin-2-yldiazomethane (I) introduces the 1-oxidopyridin-2-ylmethyl protecting group into acidic substances ($pK_a < 9{\cdot}8$) including nucleotides; it can be removed by treatment with acetic anhydride followed by methanolic ammonia.

THE development of procedures for the chemical synthesis of oligonucleotides depends to a significant extent on the design of a new protecting group with very specific properties.[1] Although diazomethane is useful for methylating reasonably acidic substances,[2] the methyl group is of no use as a protecting group, because of difficulties in its removal.[3] We report here the synthesis of 1-oxidopyridin-2-yldiazomethane (I) and its application in the protection of hydroxy-functions.†



2-Formylpyridine 1-oxide[4] was converted into the corresponding *p*-tosylhydrazone,‡ m.p. 135—137° (50%), which was treated with NaOMe (1 equiv.) at 60°. Work up[5] afforded compound (I) (30%, as $CHCl_3$ solution); λ_{max} (aq. MeOH) 557 nm; ν_{max} 2080 (N≡N⁺) and 1235 (N → O) cm⁻¹. Compound (I) reacted rapidly with AcOH in $CHCl_3$ with evolution of nitrogen to afford 1-oxidopyridin-2-ylmethyl acetate, m.p. 67—68°, quantitatively. Results for other acidic substances are in Tables 1 and 2.



(II) Base = adenine
(III) Base = uracil

In chloroform solution, (I) did not alkylate *m*-nitrophenol (pK_a 8·4), phenol (pK_a 10·0), and uridine (pK_a 9·8 and 12·34)[6] In aqueous solution, prolonged treatment (20 h; room temperature) of uridine 5′-phosphate with excess of (I) afforded the protected derivative (IV) (85%) whose enzymatic hydrolysis with venom phosphodiesterase, followed by alkaline phosphatase treatment, afforded the protected uridine (V). These results coupled with those in Table 1 indicate that in chloroform solution (I) could alkylate acidic substances with pK_a values less than *ca* 7·5.

TABLE 1

Reactions in $CHCl_3$ *solution at* 20° *for* 3 h[a]

RXH	pK_a	M.p.	Yield
p-NO_2·C_6H_4·CO_2H ..	3·4	155—157°	Quant.
$PhCO_2H$	4·2	125—126°	Quant.
PhSH	6·5	98—100°	70%
p-NO_2·C_6H_4·OH	7·1	221—223°	71%

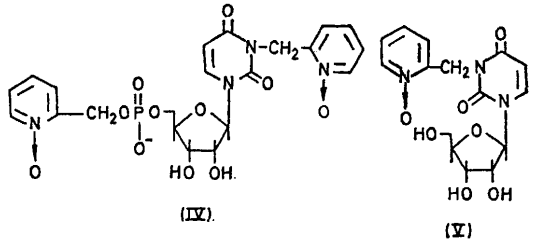
[a] Satisfactory elemental analyses were obtained for all compounds.

TABLE 2

Alkylation in aqueous solution (pH 4·5) *at* 20° *for* 2 h

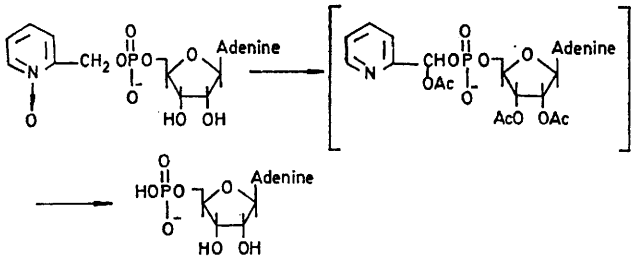
Compound	pK_a	Product	Yield
Adenosine 5′-phosphate ..	*ca.* 1	(II)[a]	84%
Uridine 5′-phosphate	*ca.* 1	(III)[b]	89%

[a] Purified by DEAE-cellulose column chromatography. Enzymatic hydrolysis of the purified product (IV) with venom phosphodiesterase afforded adenosine 5′-phosphate and 1-oxidopyridin-2-ylmethanol, ratio 1 : 1. [b] Purified by DEAE-cellulose column chromatography. The structure was established by comparison (u.v. and R_f-values on paper electrophoresis) with an authentic sample prepared by a general method (including deacetylation) from 2′,3′-di-*O*-acetyluridine 5′-phosphate and 1-oxidopyridin-2-ylmethanol with mesitylsulphonyl chloride as a condensing agent.



† The use of the 1-oxidopyridin-2-ylmethyl protecting group in polynucleotide synthesis has been discussed by Mizuno, *J. Org. Chem.*, 1972, **37**, 39.

‡ Satisfactory elemental analyses were obtained for these compounds and those with m.p.s. listed herein.



In aqueous solution, however, the critical pK_a value increases to *ca.* 10.

Deblocking of (II) could be achieved by treatment with Ac_2O at 60° for 35 h (or 20° for 4 days), followed by methanolic ammonia (saturated ammonia, room temp., overnight). Recovery of adenosine 5-phosphate was 84%.

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[5] B. Eistert, W. Kurze, and G. W. Muller, *Annalen*, 1970, **732**, 1; M. Regitz, *Chem. Ber.*, 1966, **99**, 2918.
[6] R. M. Izatt, J. H. Rytting, L. D. Hansen, and J. J. Christensen, *J. Amer. Chem. Soc.*, 1966, **88**, 2641. The absence of alkylation of uridine in chloroform may have been due to its insolubility. Alkylation in aqueous solution is now being undertaken.