[Chem. Pharm. Bull. 28(6)1695—1703(1980)]

Synthetic Nucleosides and Nucleotides. XV.[1] 5-Dimethylamino-2-oxidoisoquinolin-1-yl Diazomethane: A Novel Water-soluble Fluorescent Labelling Agent for Nucleotides

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(Received October 25, 1979)

A novel fluorescent labelling agent, 5-dimethylamino-2-oxidoisoquinolin-1-yl diazomethane (**3**) was designed and synthesized for the fluorescent labelling of the phosphate moiety of nucleotides and nucleic acids. Starting from 1-cyano-5-nitroisoquinoline (**4**), 1-carboxy-5-nitroisoquinoline (**5**) was obtained after hydrolysis with hydrochloric acid. Esterification of **5** with methanol in the presence of sulfuric acid afforded 1-methoxycarbonyl-5-nitroisoquinoline (**6**). Catalytic hydrogenation of **6** followed by treatment with formic acid–acetic anhydride gave the 5-formamido derivative (**8**). Methylation of **8** with methyl iodide in the presence of sodium hydride afforded the 5-N-methylformamido derivative (**9**). Reduction of both the ester group and formyl group with aluminum hydride followed by treatment with chloranil and acetic anhydride provided 1-acetoxymethyl-5-dimethylaminoisoquinoline (**11**). N-Oxidation of **11** with *m*-chloroperbenzoic acid followed by selective removal of the oxido group at the 5-position by reaction with carbon disulfide afforded 1-acetoxymethyl-5-dimethylaminoisoquinoline-2-oxide (**13**). After deacylation of **13**, selenium dioxide oxidation of the hydroxymethyl group followed by reaction with *p*-toluenesulfonyl hydrazide gave 5-dimethylamino-1-formylisoquinoline-2-oxide *p*-toluenesulfonyl hydrazone (**16**). On treatment of **16** with sodium ethoxide, the desired compound (**3**) was obtained. Reaction of **3** with *p*-nitrobenzoic acid gave the crystalline *p*-nitrobenzoyl ester. Treatment of uridine 5′-phosphate with **3** gave uridine 5′-(5-dimethylamino-2-oxidoisoquinolin-1-yl)methylphosphate (**17**). This labelled nucleotide was highly fluorescent, with an excitation maximum of 353 nm and an emission maximum at 523 nm. The fluorescence characteristics of **17** were compared with those of the model compound (**13**) and uridine 5′-(5-dimethylaminoisoquinolin-1-yl)methylphosphate (**18**).

Keywords——fluorescent labelling agent; fluorescent labelling of nucleotides; 5-dimethylamino-2-oxidoisoquinolin-1-yl diazomethane; synthesis; reaction with uridine 5′-phosphate; fluorescence properties; uridine 5′-(5-dimethylaminoisoquinolin-1-yl)-methyl phosphate; uridine 5′-(5-dimethylamino-2-oxidoisoquinolin-1-yl)methylphosphate

In studies of the higher structures of biologically important macromolecules, various spectroscopic methods have been employed in gaining information on the relationship between molecular structure and biological function. In particular, the application of fluorescence spectroscopy in the field of protein chemistry has been very successful.[3] However, fluorescence spectroscopy has had limited use in studies of nucleotides and nucleic acids, since only a few fluorescent nucleotides and fluorescent labelling agents for nucleotides are known.[4–11]

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7) C.H. Yang and D. Söll, *Arch. Biochem. Biophys.*, **155**, 70 (1973).
8) C.H. Yang and D. Söll, *J. Biochem.* (Tokyo), **73**, 1243 (1973).
9) C.H. Yang and D. Söll, *Biochemistry*, **13**, 3615 (1974).
10) C.H. Yang and D. Söll, *Proc. Nat. Acad. Sci. U.S.*, **71**, 2388 (1974).
11) N.J. Leonard and G.L. Tolman, *Ann. N.Y. Acad. Sci.*, **40**, 1392 (1975).

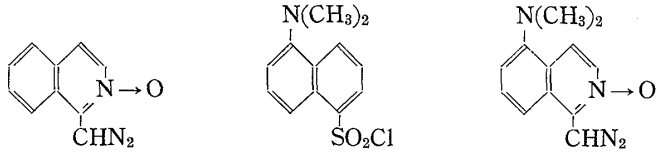
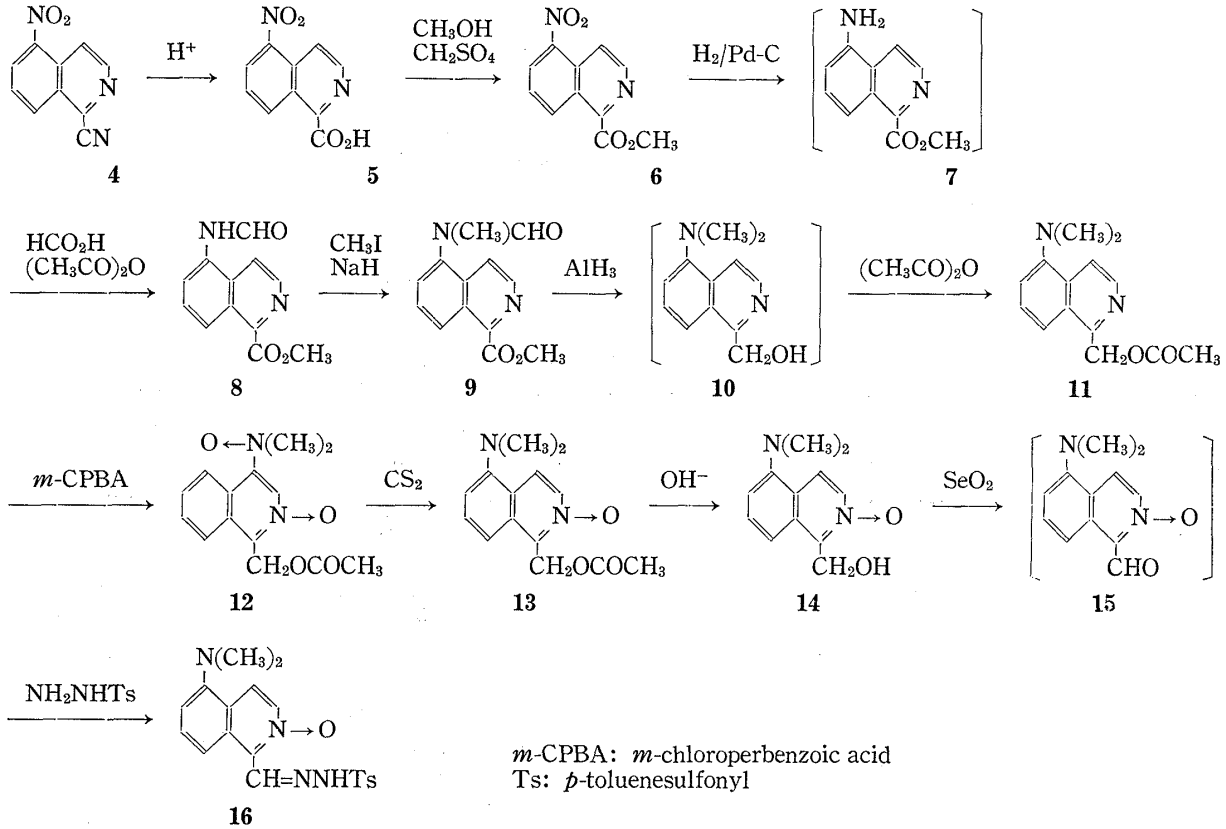


Chart 1

We now report the preparation of a new fluorescent labelling agent, 5-dimethylamino-2-oxidoisoquinolin-1-yl diazomethane (**3**), which can react selectively with the phosphate moiety of nucleotides. This labelling technique should allow fluorescent labelling of the 5′-end of transfer RNA's and should thus facilitate studies of nucleotides and transfer RNA's by fluorescent spectroscopy. Our present study was prompted by a recent report by Mizuno *et al.* that 2-oxidoisoquinolin-1-yl diazomethane (**1**) could alkylate functional groups with acidic hydrogens, such as nitrophenol, carboxylic acid or phosphates.[12] This result led us to consider that if this reagent were fluorescent, it could be utilized as fluorescent labelling agent for nucleotides. Introduction of a dimethylamino group at the 5-position of the isoquinoline nucleus seemed a promising approach in view of the structural similarity of **3** with the well known 5-dimethylaminonaphthalenesulfonyl (dansyl) group (**2**).

Synthesis of 5-Dimethylamino-2-oxidoisoquinolin-1-yl Diazomethane (3)

The synthetic scheme for **3** is shown in Chart 2. 1-Cyano-5-nitroisoquinoline (**4**)[13] prepared from isoquinoline *via* three steps, was converted to 1-carboxy-5-nitroisoquinoline (**5**) by treatment with concentrated hydrochloric acid in 93% yield. Esterification of 5 with methanol



m-CPBA: *m*-chloroperbenzoic acid
Ts: *p*-toluenesulfonyl

Chart 2

12) Y. Mizuno, T. Endo, and T. Nakamura, *J. Org. Chem.*, **40**, 1391 (1975).
13) E. Ochiai and S.R. Sai, *Yakugaku Zasshi*, **65B**, 418 (1945).

in the presence of H_2SO_4 gave 1-methoxycarbonyl-5-nitroisoquinoline (**6**), as crystals, in 94% yield. Hydrogenation of compound **6** in the presence of 5% palladium on charcoal as a catalyst at atmospheric pressure afforded 5-amino-1-methoxycarbonylisoquinoline (**7**), which was used directly for the next step without further purification. Compound **7** was treated with a 1 : 2 mixture of formic acid and acetic anhydride at room temperature to give 5-formamido-1-methoxycarbonylisoquinoline (**8**) in 72% yield. Methylation of **8** with methyl iodide in the presence of sodium hydride, followed by column chromatography on silica gel, gave 5-N-methyl-N′-formamido-1-methoxycarbonylisoquinoline (**9**) in 82% yield. Reduction of both the formyl and methoxycarbonyl groups of **9** with several reducing reagents such as $LiAlH_4$, $LiBH_4$, B_2H_6 or $NaAlH_2(OCH_2CH_2OCH_3)_2$ was unsuccessful. However, the use of AlH_3 as a reducing reagent gave the desired result. Thus, reaction of **9** with AlH_3 reduced the formyl and methoxycarbonyl groups to methyl and hydroxymethyl groups, respectively, to give 5-dimethylamino-1-hydroxymethylisoquinoline (**10**). A small amount of the 1,2-dihydro derivative of **10** was also formed in this reaction. Therefore, **10** was isolated as its acetate, 1-acetoxymethyl-5-dimethylaminoisoquinoline (**11**), after oxidation of the product mixture with chloranil in order to convert the 1,2-dihydro derivative to **10**. The yield of compound **11** was 54% based on the amount of **9**. The structure of **11** was assigned from its nuclear magnetic resonance (NMR) spectrum, which showed the N-methyl signal at δ 2.86 and a signal at δ 5.67 corresponding to the methylene group at the 1-position of the isoquinoline nucleus. N-oxidation of **11** was performed by treatment with *m*-chloroperbenzoic acid to give 1-acetoxymethyl-5-dimethylaminoisoquinoline-2,5-dioxide (**12**) in 47% yield. Structural assignment of **12** was based on its NMR and infrared (IR) spectra. In the NMR spectrum, the methyl signal of the dimethylamino group was shifted to δ 3.90, suggesting the introduction of N-oxide at the 5-position. The IR absorption maxima at 1280 cm^{-1} and 940 cm^{-1} are characteristic of aromatic N-oxide and aliphatic N-oxide, respectively. The selective removal of N-oxide from the 5-position was carried out by treatment with carbon disulfide in methanol[14] to give 1-acetoxymethyl-5-dimethylaminoisoquinoline-2-oxide (**13**) in 70% yield. The methyl signal of this compound was shifted back to the original high field (δ 2.89) in the NMR spectrum and the IR absorption maximum at 940 cm^{-1} disappeared, with that at 1280 cm^{-1} still remaining. Treatment of **13** with ethanolic ammonia followed by column chlromatography on silica gel afforded 5-dimethylamino-1-hydroxymethylisoquinoline-2-oxide (**14**) as yellow crystals in 88% yield. Oxidation of **14** with SeO_2 in pyridine afforded an aldehyde (**15**) which in turn, was treated with *p*-toluenesulfonyl hydrazide to give a crystalline hydrazone (**16**) in 68% yield. The final yield of **16** from **4** was 5.5%. The desired diazomethane (**3**) could be obtained by treatment of **16** with sodium ethoxide, followed by extraction with benzene. The diazomethane (**3**) thus obtained was used as its dioxane solution. Compound **3** reacted with *p*-nitrobenzoic acid to give crystalline *p*-nitrobenzoate. This compound was identical with an authentic specimen prepared by an alternative synthesis from **10** (mixed melting point and IR spectra).

Preparation of Uridine 5′-(5-Dimethylamino-2-oxidoisoquinolin-1-yl)methylphosphate (17) and Uridine 5′-(5-Dimethylaminoisoquinolin-1-yl)methylphosphate (18)

The newly prepared 5-dimethylamino-2-oxidoisoqiunolin-1-yl diazomethane (**3**) was treated with uridine 5′-phosphate in aqueous solution at pH 5.0, followed by purification with paper electrophoresis and paper chromatography to give **17**. This compound was homogeneuos in paper chromatography and paper electrophoresis. The structure was confirmed by enzymatic hydrolysis of **17** with snake venom phosphodiesterase. The product (**17**) was highly fluorescent.

To assist in achieving an understanding of the spectral properties of **17**, compound **18**, which has no N-oxide group at the 2-position of the isoquinoline nucleus, was prepared by

14) J.S. Wieczorek and E. Plazek, *Rec. Trav. Chim.*, **84**, 785 (1965).

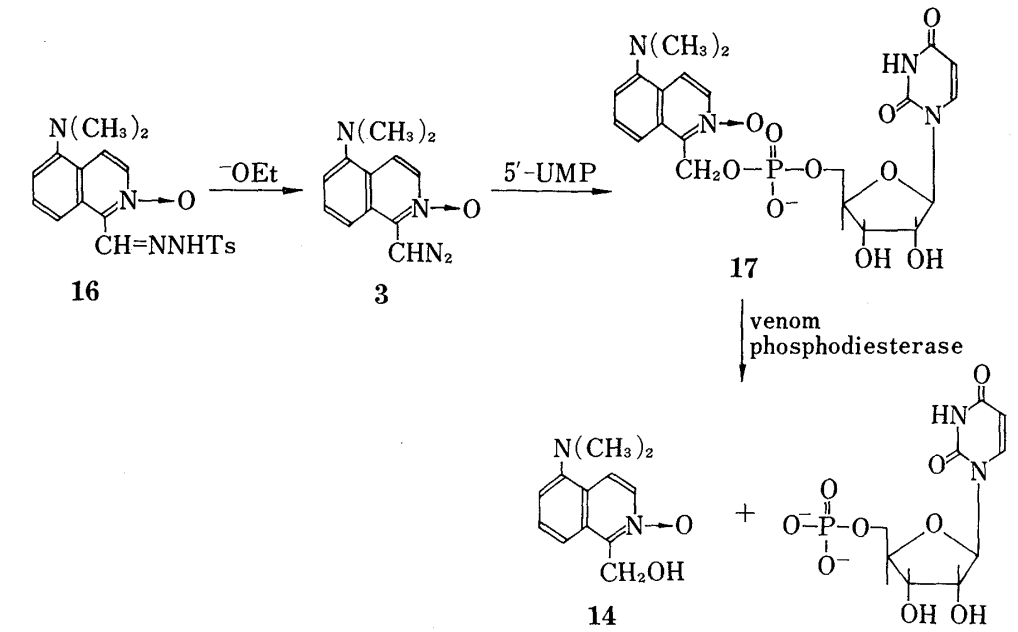
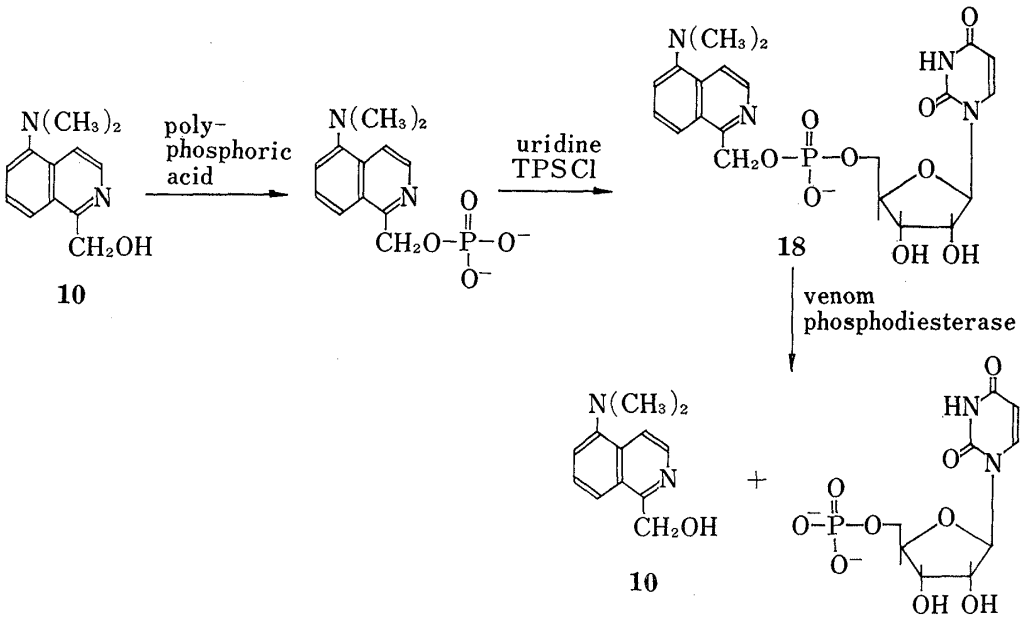


Chart 3



TPSCl: triisopropylbenzenesulfonyl chloride

Chart 4

following route. 5-Dimethylamino-1-hydroxymethylisoquinoline (**10**) was phosphorylated with polyphosphoric acid, followed by purification with diethylaminoethyl (DEAE)-cellulose column chromatography. The resulting 5-dimethylaminoisoquinolin-1-yl methylphosphate was coupled with uridine in pyridine in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride as a condensing agent. The product was purified by preparative paper electrophoresis. A small amount of contaminating 2′(3′)-isomers was removed by paper chromatography. The product thus obtained was homogeneous in paper chromatography and paper electrophoresis. The structure of **18** was confirmed by enzymatic hydrolysis with snake venom phosphodiesterase. Compound **18** was hydrolyzed to uridine 5′-phosphate and **10**.

Ultraviolet (UV) Absorption and Fluorescence Properties of Compounds bearing the 5-Dimethylaminoisoquinoline Moiety

As can be seen in Fig. 1, the fluorescence spectra of 1-acetoxymethyl-5-dimethylaminoisoquinoline-2-oxide (**13**) as a model compound of a nucleotide ester, in aqueous dioxane depended on the water content. Maximum fluorescence intensity of emission appeared in 100% dioxane solution at 470 nm, with excitation at 302 nm. The excitation and emission maxima were both shifted to longer wavelength with increasing water content. At the same time the fluorescence intensity was strongly quenched. The relative fluorescence intensity was 100 in dioxane and 1 in water. These results are similar to those for other usual fluorescent compounds, such as dansyl amino acids. UV data for **13**, **14**, **17** and **18** are shown in Table I. The pK_a value of compound **14** was calculated to be 2.7 according to the method of Shugar and Fox.[15] The fluorescence spectrum of the acetylated derivative **13**, which is a model com-

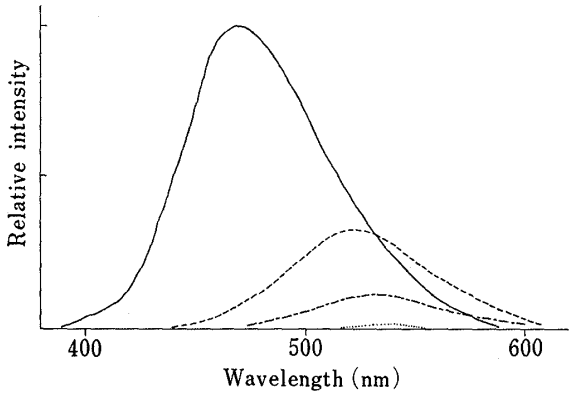


Fig. 1. Changes of the Fluorescence Intensities of 1-Acetoxymethyl-5-dimethylaminoisoquinoline-2-oxide (**13**) in Mixtures of Dioxane and Water

	dioxane	water (%)
———,	100	0
– – – –,	70	30
— — —,	40	60
·····,	0	100

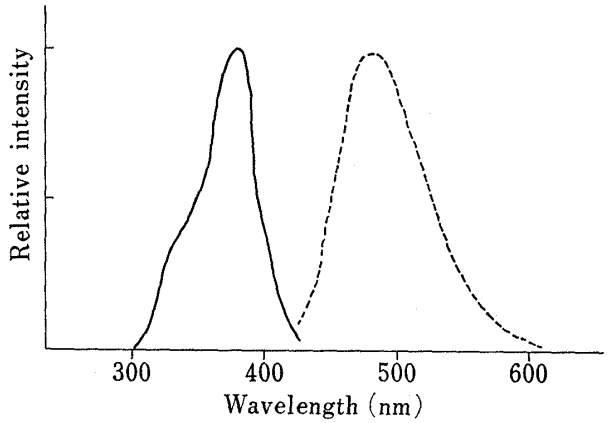


Fig. 2. Fluorescence Spectrum of 1-Acetoxymethyl-5-dimethylamino-2-oxidoisoquinoline (**13**)

———, excitation spectrum,
– – – –, emission spectrum.

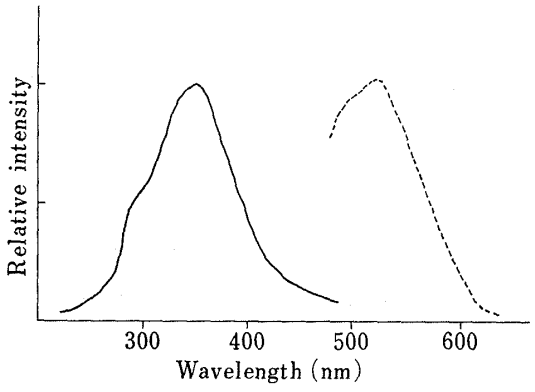


Fig. 3. Fluorescence Spectrum of Uridine 5′-(5-Dimethylamino-2-oxidoisoquinolin-1-yl)methylphosphate (**17**)

———, excitation spectrum,
– – – –, emission spectrum.

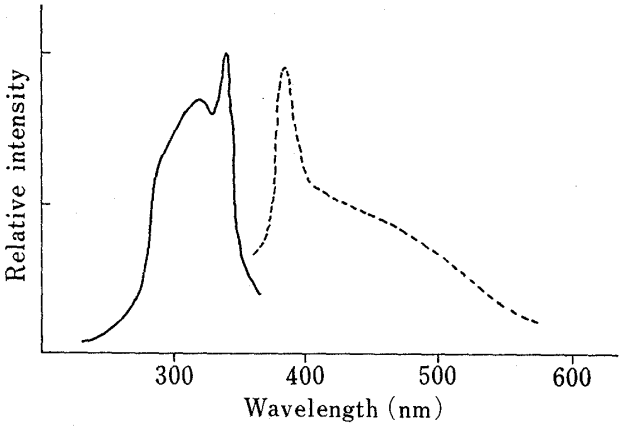


Fig. 4. Fluorescence Spectrum of Uridine 5′-(5-Dimethylaminoisoquinolin-1-yl)methylphosphate (**18**)

———, excitation spectrum,
– – – –, emission spectrum.

15) D. Shugar and J.J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952).

pound for the labelled nucleotide ester (**17**), is shown in Fig. 2. Compared with the fluorescence spectrum of **14**, its excitation maximum was shifted to longer wavelength (380 nm) and the emission maximum was shifted to 482 nm (Table I). On the other hand, the fluorescence characteristics of the uridylic acid derivative (**17**), as shown in Fig. 3, were more significantly changed. The excitation maximum was shifted to shorter wavelength (353 nm) and the emission maximum, in contrast, was shifted to 523 nm. The wavelength difference between the excitation and emission maxima of compound **17** was 170 nm.

In order to clarify the structure-fluorescence property relationships, the fluorescence spectrum of compound **18**, which has no oxido group at position 2 of the isoquinoline ring, was measured under the same conditions. The fluorescence spectrum is shown in Fig. 4, and is quite different from that of the corresponding N-oxide (see Fig. 3). The excitation maximum was at 338 nm and the emission maximum was 383 nm, *i. e.*, markedly shifted to shorter wavelength. The wave length difference was only 50 nm. These characteristics are summarized in Table I.

Table I. UV and Fluorescence Characteristics of Various 5-Dimethylaminoisoquinoline Derivatives

Compound	Solvent	UV, λ_{max} (nm)	Ex. max (nm)	Em. max (nm)
5-Dimethylamino-1-hydroxymethyl-isoquinoline-2-oxide (**14**)	Dioxane	253, 297	302, 335	470
	Water	223, 241, 278	295, 345	545
1-Acetoxymethyl-5-dimethylamino-isoquinoline-2-oxide (**13**)	Dioxane	250, 297	380	482
Uridine 5′-(5-dimethylamino-2-oxidoisoquinolin-1-yl)methylphosphate (**17**)	Water	244, 267	353	53
Uridine 5′-(dimethylamino isoquinolin-1-yl)methylphosphate (**18**)	Water	250, 325	338	383

In conclusion, the 5-dimethylamino-2-oxidoisoquinoline nucleus shows strong fluorescence and the wavelength difference between the excitation maximum and emission maximum is more than 100 nm. These results suggest that the fluorescent labelling agent, 5-dimethylamino-2-oxidoisoquinolin-1-yl diazomethane (**3**) is suitable for use with nucleotides and nucleic acids. Compound **3** was fairly soluble in aqueous media containing 10% dioxane. The half-life of labelled nucleotide (**17**) was examined in appropriate buffer solutions using paper electrophoresis and UV spectroscopic analysis. Incubation was performed at 37°. The values obtained were 24 hr at pH 7.1, 12 hr at pH 10.1 and 7 hr at pH 4.1.

Application of this newly prepared fluorescent labelling agent (**3**) to other nuclotides and transfer RNA's will be the subject of a future report.

Experimental

Melting points were determined on a Yanaco melting point apparatus and are uncorrected. NMR spectra were obtained on Hitachi R20B high resolution NMR spectrophotometer with tetramethylsilane as an internal standard. UV spectra were recorded on a Shimadzu UV-300 recording spectrophotometer. The fluorescence spectra were obtained on a Hitachi fluorescence spectrometer. Snake venom phosphodiesterase from Russell's viper was purchased from C.F. Boehringer and Sohne GmbH, Mannheim (Germany). Paper chromatography was performed by the ascending technique using Toyo Filter Paper No. 51A. The solvent systems used were: A, *n*-butanol–acetic acid–water (4: 1: 2, v/v/v); B, 2-propanol–conc. NH_4OH–water, (7: 1: 2, v/v/v). Thin-layer chromatography was performed by the ascending technique using Avicel SF cellulose plate.

1-Carboxy-5-nitroisoquinoline (**5**)——A solution of 1-cyano-5-nitroisoquinoline (**4**)[13] (10.0 g) in concentrated hydrochloric acid (300 ml) was heated at 100° for 5 hr. On standing at room temperature, light-yellow crystals precipitated. The crystals were collected by filtration and dried. 10.2 g (93%). mp 168—169°. *Anal.* Calcd for $C_{10}H_6N_2O_4$: C, 55.05; H, 2.77; N, 12.84. Found: C, 55.11; H, 2.80; N, 12.87. MS *m/e*: 218 (M^+). IR, ν_{max}^{KBr} (cm^{-1}): 1718 (C=O), 1520 and 1335 (NO_2).

1-Methoxycarbonyl-5-nitroisoquinoline (**6**)——A total of 50 ml of concentrated sulfuric acid was added carefully in several portions to a suspension of 1-carboxy-5-nitroisoquinoline (**5**) (10.0 g) in methanol (500 ml). After refluxing for 3 hr, the solution was concentrated to a small volume under reduced pressure. Distilled water (200 ml) was added to the residue and the pH of the mixture was adjusted to 3 with sodium bicarbonate. The turbid mixture was extracted with chloroform (100 ml×3). The combined extracts were dried over magnesium sulfate and concentrated. The residue thus obtained was crystallized from hot ethyl acetate to give **6** as light-yellow needles. 10.0 g (94%). mp 162—163°. *Anal.* Calcd for $C_{11}H_8N_2O_4$: C, 56.90; H, 3.47; N, 12.06. Found: C, 56.78; H, 3.51; N, 11.93. MS *m/e*: 232 (M^+). IR, ν_{max}^{KBr} (cm^{-1}): 1720 (C=O).

5-Formamido-1-methoxycarbonylisoquinoline (**8**)——1-Methoxycarbonyl-5-nitroisoquinoline (**6**) (11 g) was dissolved in ethanol (400 ml) and hydrogenated at room temperature under atmospheric pressure with 5% palladium on charcoal (3.3 g) as a catalyst. After absorption of hydrogen had ceased, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The resulting crude 5-amino-methoxycarbonylisoquinoline (**7**) was treated with a mixture of formic acid and acetic anhydride (40 ml: 80 ml) and the reaction mixture was stirred overnight at room temperature. The solution was evaporated to dryness and the residue was dissolved in distilled water (10 ml). The solution was added to saturated aqueous sodium carbonate to yield a precipitate, which was collected by filtration and washed well with water. After being air-dried, this material was crystallized from methanol to give **8** as colorless needles. 7.9 g (72%). mp 185—187°. *Anal.* Calcd for $C_{12}H_{10}N_2O_3$: C, 62.61; H, 4.38; N, 12.18. Found: C, 62.67; H, 4.38; N, 12.43. MS *m/e*: 230 (M^+). IR: ν_{max}^{KBr} (cm^{-1}); 1730 (ester C=O), 1700 (amide C=O).

5-N-Methyl-N′-formylamido-1-methoxycarbonylisoquinoline (**9**)——Sodium hydride (1.36 g) was added to a solution of 5-formamido-1-methoxycarbonylisoquinoline (**8**) (4.31 g) in anhydrous tetrahydrofuran (600 ml), and the mixture was heated under reflux for 30 min. After cooling to room temperature, the solution was treated with methyl iodide (1.77 ml) and heated under reflux again for 3 hr. The solution was concentrated to 200 ml and mixed with silica gel (10 g). The mixture was then evaporated to dryness and the residue was applied to a column of silica gel (200 g). Elution was performed with chloroform–acetone (4: 1). The eluates were combined and concentrated. The residue was crystallized from ethyl acetate and *n*-hexane to give **9** as colorless needles. 3.73 g (82%). mp 108—109°. *Anal.* Calcd for $C_{13}H_{12}N_2O_3$: C, 63.93; H, 4.95; N, 11.47. Found: C, 63.95; H, 4.90; N, 11.49. MS *m/e*: 244 (M^+), NMR: δ 3.37 ($N-CH_3$).

1-Acetoxymethyl-5-dimethylaminoisoquinoline (**11**)——Compound (**9**) (0.98 g) was dissolved in anhydrous tetrahydrofuran (50 ml) and the solution was kept an ice-water mixture. Aluminum hydride, prepared from $LiAlH_4$ (0.51 g) by reaction with concentrated sulfuric acid in tetrahydrofuran (50 ml), was added dropwise to the solution. After stirring for 1 hr, the reaction mixture was heated under reflux for a further 2 hr. Distilled water (20 ml) was added gradually to the mixture to decompose excess aluminum hydride and the complex. Acetone (30 ml) was added to the mixture and the insoluble material was filtered off. The filtrate and washings were combined and concentrated. The residue was treated with water (20 ml) and extracted with ethyl acetate (20 ml×3). The combined extracts, which contained 5-dimethylamino-1-hydroxymethylisoquinoline (**10**), were dried over magnesium sulfate, then chloranil (1.97 g) was added. The reaction mixture was stirred for 10 min at room temperature and evaporated to dryness under reduced pressure. Acetic anhydride (10 ml) was added to the residue and the mixture was allowed to stand at room temperature overnight. This mixture was treated with aqueous ethanol and concentrated. The oily residue was mixed with saturated aqueous sodium bicarbonate (30 ml) and the mixture was extracted with chloroform (10 ml×3). The chloroform extracts were dried over sodium sulfate and concentrated. The residue was applied to a column of silica gel (100 g) with chloroform–ethyl acetate (4: 1, v/v) as an eluent. The eluate was concentrated to give **11** as a sticky gum which was pure as determined by TLC analysis. 0.53 g (54%). MS *m/e*: 244 (M^+). IR: ν_{max}^{KBr} (cm^{-1}), 1740 ($-OCO-CH_3$). NMR (MeOH-d_4): δ 2.11 (ester CH_3), 2.86 (5-diCH_3), 5.67 ($-CH_2-$).

1-Acetoxymethyl-5-dimethylaminoisoquinoline-2,5-dioxide (**12**)——Compound **11** (0.34 g) was treated with *m*-chloroperbenzoic acid (1.40 g) in methanol (20 ml) under stirring for 5 hr at room temperature. After removing the solvent by evaporation, the residue was dissolved in a small amount of water and extracted with ether to remove *m*-chlorobenzoic acid. The aqueous phase was evaporated down, applied to a column of alumina (20 g), and eluted with chloroform–methanol (19: 1, v/v). The fractions which contained the desired product were combined and concentrated. The resulting glassy residue was crystallized from methanol–acetone–*n*-hexane mixture to give **12** as colorless needles. 0.18 g (47%). mp 171—173°. *Anal.* Calcd for $C_{14}H_{16}N_2O_4$: C, 60.86; H, 5.84; N, 10.14. Found: C, 61.09; H, 6.05; N, 10.03. MS *m/e*: 260 (M-16). IR: ν_{max}^{KBr} (cm^{-1}), 1280 (aromatic N-oxide), 940 (aliphatic N-oxide). NMR (MeOH-d_4), δ 3.90 (5-diCH_3).

1-Acetoxymethyl-5-dimethylaminoisoquinoline-2-oxide (**13**)——Carbon disulfide (20 ml) was added to a solution of compound **12** (0.96 g) in methanol (20 ml), and the mixture was heated under reflux for 1 hr. The solvent was removed under reduced pressure and the residue was dissolved in a small amount of chloroform. The solution was applied to a column of alumina (50 g) with chloroform–methanol (19: 1, v/v), and the eluate was concentrated to give 13 as a TLC homogeneous gum. 0.63 g (70%). MS *m/e*: 244 (M-16). NMR (MeOH-d_6), δ 2.89 (5-diCH_3).

5-Dimethylamino-1-hydroxymethylisoquinolin-2-oxide (**14**)——A solution of compound **13** (0.50 g) in a mixture of ethanol and concentrated ammonia (50 ml: 50 ml) was stirred for 1 hr. After removal of the

solvent, the residue was chromatographed on a silica gel column (10 g) with chloroform–methanol (19: 1, v/v) as an eluting solvent. The eluate was evaporated to dryness and the residue was crystallized from a mixture of acetone, ethyl acetate and *n*-hexane to give **14** as bright-yellow needles. 0.37 g (88%). mp 136—137°. *Anal.* Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.14; H, 6.53; N, 12.89. MS *m/e*: 218 (M^+). UV: λ_{max} (nm), 223 (ε=22300), 241 (19900), 278 (ε=21000) and 320 (shoulder). $\lambda_{max}^{0.01N\ HCl}$ (nm), 223, 256, 287 and 297.

5-Dimethylamino-1-formylisoquinoline-2-oxide *p*-Toluenesulfonyl Hydrazone (16)——Selenium dioxide (38 mg) was added to a solution of compound **14** (0.15 g) in pyridine (5 ml) and the mixture was refluxed for 5 hr. The solvent was evaporated off and the residue was mixed with benzene (20 ml). The insoluble material was removed by filtration through celite column (10 g). The filtrate containing 5-dimethylamino-1-formylisoquinoline-2-oxide (**15**), was treated with *p*-toluenesulfonyl hydrazide (0.15 g) in benzene (50 ml), and the reaction mixture was allowed to stand at room temperature for 1 hr. The reaction mixture was then concentrated to 20 ml to yield **16** as orange-red crystal. 0.18 g (68%). mp 126—128° (dec.). *Anal.* Calcd for $C_{19}H_{20}N_4O_3S$: C, 59.36; H, 5.24; N, 14.57; S, 8.34. Found: C, 59.30; H, 5.19; N, 14.49; S, 8.20. IR: ν_{max}^{KBr} (cm^{-1}), 1160 and 1330 ($-SO_2-$), 1215 (aromatic N-oxide).

Preparation of 5-Dimethylamino-2-oxidoisoquinolin-1-yl Diazomethane (3) from Compound 16——Compound **16** (19 mg) was added to 0.1 N sodium ethoxide in ethanol (0.5 ml). The mixture was stirred continuously at room temperature for 30 min and then at 60°. When the spot on TLC (silica gel, chloroform–methanol=19: 1, v/v) corresponding to **16** had disappeared and a new spot was detected, the reaction mixture was allowed to cool to room temperature then concentrated. The residue was dissolved in a small volume of benzene. Insoluble material was removed by filtration. The filtrate was then evaporated down and the residue (**3**) was dissolved in anhydrous dioxane for use.

5-Dimethylamino-1-*p*-nitrobenzoyloxymethylisoquinoline-2-oxide——Method A: 5-Dimethylamino-1-hydroxymethylisoquinoline (**10**) prepared from **11** (0.10 g) was mixed with *p*-nitrobenzoyl chloride (91 mg) in anhydrous pyridine (5 ml). The reaction mixture was stirred at room temperature overnight then concentrated. Distilled water was added to the residue, which was then evaporated down again. This process was repeated three times. The residue was dissolved in chloroform (20 ml) and washed with saturated aqueous sodium bicarbonate to remove *p*-nitrobenzoic acid. After washing with water, the chloroform layer was dried over magnesium sulfate and concentrated. The residue was dissolved in methanol (20 ml) with *m*-chloroperbenzoic acid (0.42 g) and the reaction mixture was stirred for 10 hr at room temperature. The solvent was removed under reduced pressure and the residue was redissolved in distilled water. The solution was extracted with ether to remove *m*-chlorobenzoic acid. The aqueous phase was evaporated to dryness and the residue was treated with carbon disulfide (10 ml) in methanol (10 ml). After refluxing for 1 hr, the solvent was removed *in vacuo*. The product was purified by preparative thin-layer chromatography on silica gel using chloroform–methanol (39: 1, v/v) as a developing solvent. The main band, which corresponded to the desired product, was cut off and eluted with acetone. The solvent was evaporated off and the residue was crystallized from acetone and *n*-hexane to give a pure crystalline material. 42 mg (28%). mp 172—175°. *Anal.* Calcd for $C_{19}H_{17}N_3O_5$: C, 62.12; H, 4.66; N, 11.44. Found: C, 61.98; H, 4.49; N, 11.33. IR: ν_{max}^{KBr} (cm^{-1}), 1720 (C=O), 1527 and 1345 ($-NO_2$), 1275 (aromatic N-oxide).

Method B: Compound **3** prepared from **16** (38 mg) in dioxane (10 ml) was treated with *p*-nitrobenzoic acid (18 mg) and the reaction mixture was stirred for 3 hr at room temperature. After removal of the solvent by evaporation and purification of the residue by preparative thin-layer chromatography as described in Method A, the final residue was crystallized from acetone and *n*-hexane to give crystals. 12 mg (33%). mp 169—172°. This sample was identified as 5-dimethylamino-1-*p*-nitrobenzoyloxymethylisoquinoline-2-oxide on the basis of the mixed fusion test and comparison of its IR spectrum with that of the sample obtained by Method A.

Uridine 5′-(5-Dimethylamino-2-oxidoisoquinolin-1-yl)methylphosphate (17)——Uridine 5′-phosphate (disodium salt, 9 mg) in 1 ml of distilled water, the pH of which was adjusted to 5 with hydrochloric acid, was added to a solution of **3** prepared from 4.8 mg of **16** in 1 ml of dioxane. The resulting clear solution was stirred for 20 min at room temperature and was concentrated to a small volume. This was applied to a Whatman 3MM paper sheet. Electrophoresis was performed in 0.05 M triethylammonium bicarbonate (pH 8.0) at 700 V for 1.5 hr. The main band (R_{UMP}=0.59) corresponding to the diester was cut off and eluted with the same buffer. This product was homogeneous in thin-layer chromatography (Rf=0.53 in solvent A and 0.55 in solvent B), appearing as a bright yellow fluorescent spot. UV: $\lambda_{max}^{H_2O}$ (nm), 244 and 267; $\lambda_{max}^{0.01N\ NaOH}$ (nm); 245 (shoulder) and 270. $\lambda_{max}^{0.01N\ HCl}$ (nm); 257, 298 (shoulder) and 312 (shoulder). Compound **17** (0.88 optical density unit at 260 nm) was digested in 100 μl of 0.05 M sodium acetate (pH 8.0) by the addition of snake venom phosphodiesterase (10 μg as protein). This solution was incubated at 37° for 3 hr. After separation by paper electrophoresis at 700 V for 1.5 hr, the ratio of uridine 5′-phosphate to compound **14** was calculated from their molar extinction coefficients (ε) and was found to be 1: 1.07.

Uridine 5′-(5-Dimethylaminoisoquinolin-1-yl)methylphosphate (18)——5-Dimethylamino-1-hydroxymethylisoquinoline (**10**) derived from **11** (0.45 g) was mixed with polyphosphoric acid (P_2O_5, 2.25 g and 85% phosphoric acid, 3.00 g) and the mixture was heated at 70° for 48 hr. After cooling to room temperature, the reaction mixture was added to water (20 ml) and the whole was heated again in a boiling water-bath

for 1 hr. Activated charcoal (10 g) was added to the reaction mixture and the whole was stirred for 1 hr. The charcoal was filtered off and the filter cake was washed well with water until the filtrate became neutral. The washed charcoal was suspended in a mixture of distilled water (400 ml), benzene (50 ml), methanol (200 ml) and triethylamine (50 ml). After stirring the mixture for 2 hr at room temperature, the charcoal was removed by filtration through celite and the filtrate was concentrated *in vacuo*. The residue was dissolved in a small amount of distilled water and the solution was applied to a column of diethylaminoethyl (DEAE)-cellulose (3.2 cm × 30 cm, bicarbonate form). After washing with 0.01 M triethylammonium bicarbonate (pH 8.0, 500 ml), elution was performed with a linear gradient from 0.01 M triethylammonium bicarbonate (pH 8.0, 1.2 l) to 0.3 M of the same buffer (1.2 l). Twenty ml fractions were collected. Fractions No. 37 to 65 were combined and concentrated. Water was added to the residue and evaporated off again to remove triethylamine, yielding the triethylammonium salt of 5-dimethylaminoisoquinolin-1-yl methyl phosphate, 0.24 g (33%). Paper electrophoretic mobility at 700 V, 50 min in 0.05 M triethylammonium bicarbonate (pH 8.0): $R_{GMP}=0.88$. UV: $\lambda_{max}^{H_2O}$ (nm); 211, 251 and 332. Thin-layer chromatography: $Rf=0.64$ (A) 0.49 (B). The resulting ester (26 mg) was dissolved in anhydrous pyridine (5 ml) containing 2,4,6-triisopropylbenzenesulfonyl chloride (81 mg) and the solution was stirred for 20 min at room temperature. Dried uridine (33 mg) was then added. After stirring the reaction mixture for 3 hr at room temperature, distilled water (10 ml) was added to degrade excess reagent. After 20 min, the solvent was evaporated off and the residue was re-dissolved in distilled water (1.4 ml). A 100 μl aliquot of this solution was applied to three sheets of Whatman 3MM paper. Electrophoresis was performed in 0.05 M triethylammonium bicarbonate (pH 8.0) at 700 V for 1.5 hr. The band ($R_{UMP}=0.63$) corresponding to the diester was cut off and eluted with water. This product contained the expected 5′-phosphodiester and 2′(3′)-isomers in a ratio of 2:1 as determined by paper chromatography. It was then further purified by paper chromatography on Whatman 3 MM paper sheets. Development was done by the descending technique with *n*-butanol–acetic acid–water (4:1:2, v/v/v) as the solvent system. The product showed two clear bands ($Rf=0.50$ and 0.60) on the chromatogram. The spot corresponding to $Rf=0.50$ was identified as the 5′-phosphodiester by enzymatic hydrolysis. This compound was homogeneous on paper electrophoresis ($R_{UMP}=0.63$, pH 8.0, 700 V for 1.5 hr) and thin-layer chromatography ($Rf=0.50$ in solvent A, $Rf=0.63$ in solvent B). UV, $\lambda_{max}^{H_2O}$ (nm); 250 (shoulder) and 325. $\lambda_{max}^{0.01N\ HCl}$ (nm); 256 and 345. $\lambda_{max}^{0.01N\ NaOH}$ (nm); 250 (shoulder) and 326.

The resulting compound **18** was digested in 0.05 M sodium acetate buffer (pH 8.0, 100 μl) with snake venom phosphodiesterase for 3 hr. The product was separated by paper electrophoresis (700 V for 1 hr) to give a spots corresponding to uridine 5′-phosphate and compound **10** ($R_{UMP}=0.12$). In contrast, the 2′(3′)-isomer was completely resistant to digestion under these conditions.

pH Stability of Compound 17 and 18——Compound **17** was incubated in 0.1 M sodium acetate (pH 4.1) or 0.03 M Phosphate buffer (pH 7.1) or 0.05 M sodium carbonate buffer (pH 10.1 at 37°). UV absorption spectra were measured from zero time of incubation up to 4, 8, 18 and 48 hr. Aliquots were removed from the incubation mixture at the times described above and the remaining **17** was analyzed by paper electrophoresis at 700 V for 1 hr. After 48 hr, compound **17** was cleaved completely to uridine 5′-phosphate and compound **14** at any pH examined. The half-life of compound **17** at different pHs was calculated from the difference UV spectra at each pH. The values obtained were 7, 12 and 24 hr at pH 4.1, pH 10.1 and pH 7.1, respectively. In contrast, compound **18** was found to be quite stable under these conditions.