(lit.[28] mp 157° by a different method); nmr (DMSO-d_6) 7.46 ppm (s, 1, HC=N), 7.63 (d, J = 9 Hz, 2, Ar), 7.97 (d, J = 9 Hz, 2, Ar), 11.82 (s, 1, OH).

Reaction of *anti*-*p*-Bromobenzaldoxime (30).—A mixture of **30** (2.74 g), DCC (4.5 g), and TFA (0.20 ml) was reacted for 2.5 hr in DMSO (20 ml) and benzene (20 ml). After the usual work-up sublimation at 50° (0.1 mm) gave 2.10 g (84%) of nitrile **29** identical with that above. The residue (400 mg) contained a roughly 5:3 mixture of *p*-bromobenzaldehyde and nitrone **32** by quantitative tlc but isolation was not attempted.

Alkylation of *p*-Bromobenzaldoxime with Chloromethyl Methyl Sulfide. A.—Pentane washed sodium hydride (65 mg) was added to a stirred solution of the *syn* oxime (**27**) (417 mg) in benzene (20 ml). After 10 min at 25° the mixture was heated to 70° for 10 min, and cooled while chloromethyl methyl sulfide (1 ml) was added in several portions. After 15 min at 50° the yellow solution was filtered and evaporated *in vacuo* leaving 520 mg of a yellow oil which was purified by chromatography on a column of silicic acid. Elution with benzene–chloroform (4:1) gave 225 mg (42%) of *O*-(thiomethoxymethyl)-*p*-bromobenzaldoxime (**32**) which was sublimed at 70° (0.1 mm) with mp 61–62°: λ_{max}^{MeOH} 267 mμ (ε 19,100); 298 (3400); nmr ($CDCl_3$) 2.27 ppm (s, 3, SCH_3), 5.24 (s, 2, OCH_2S), 7.45 (s, 4, Ar), 8.04 (s, 1, HC=N).

Anal. Calcd for $C_9H_{10}NOSBr$: C, 41.55; H, 3.88; N, 5.39; S, 12.33. Found: C, 41.73; H, 3.97; N, 5.42; S, 12.57.

Traces (45 mg total) of unreacted oxime and *p*-bromobenzaldehyde were eluted with chloroform–ethyl acetate (1:1) and 217 mg (40%) of nitrone (**32**) was obtained with chloroform–ethyl acetate (1:5). After sublimation at 90° (0.1 mm) this material was identical with **32** obtained from the DMSO–DCC reaction.

B.—A reaction identical with A was carried out except that the *anti* oxime (**30**) was used. The products were identical with those from the *syn* oxime by melting point and by nmr and ir spectra.

Reaction of *N*-Phenylhydroxylamine (33).—Trifluoroacetic acid (0.075 ml, 1 mmol) was added to a solution of freshly prepared *N*-phenylhydroxylamine[22] (1.11 g, 10 mmol) and DCC (6.0 g, 29 mmol) in a mixture of DMSO (15 ml) and benzene (15 ml). The initially pale yellow solution rapidly became green, then yellow, and finally red. After 14 hr the mixture was diluted with benzene and excess DCC was destroyed by addition of oxalic acid (20 mmol). After filtration, the solution was extracted three times with water, dried, and evaporated leaving 1.06 g of an oil that was chromatographed on a column containing 100 g of silicic acid. The major product (540 mg, 54%) was eluted with a gradient of chloroform in benzene (20–80%) and was followed by small amounts of four unidentified compounds. The product crystallized upon storage giving *trans*-azoxybenzene as yellow needles of mp 34.5–35.5° (lit.[29] mp 36°): λ_{max}^{MeOH} 230 mμ (ε 9100), 259 (7800), 320 (14,700) (virtually identical with lit.[30] values); nmr ($CDCl_3$) 7.23–7.66 (m, 6, Ar), 8.01–8.41 (m, 4, Ar).

Reaction of *N*,*N*-Dibenzylhydroxylamine (35).—A solution of *N*,*N*-dibenzylhydroxylamine (2.13 g, 10 mmol), DCC (4.2 g, 20 mmol), and trifluoroacetic acid (0.1 ml, 1.3 mmol) in DMSO (10 ml) and ether (10 ml) was kept at 25° for 1 hr. The mixture was then diluted with chloroform, filtered, extracted several times with water, dried (Na_2SO_4), and evaporated leaving a clear syrup. Crystallization from benzene gave 1.76 g (84%) of α-phenyl-*N*-benzylnitrone (**38**) with mp 81–83° (lit.[31] mp 82–83°): λ_{max}^{MeOH} 294 mμ (ε 20,300), 222 sh (9800); nmr ($CDCl_3$) 5.03 ppm (s, 2, $ArCH_2N$), 7.2–7.6 (m, 9, Ar), 8.1–8.4 (m, 2, Ar and HC=N); ir (KBr) 1590 cm^{-1}.

Chromatography of the mother liquors in silicic acid gave benzaldehyde (310 mg, 10% as the 2,4-dinitrophenylhydrazone) and a small amount of *N*-benzylbenzamide of mp 102–104° which was identical in every way with an authentic sample.

Registry No.—**5**, 19133-01-8; **6**, 25056-49-9; **14**, 25056-50-2; **15**, 25055-75-8; **17**, 25055-76-9; **18**, 25055-77-0; **20**, 20678-99-3; **21**, 25062-42-4; **22b**, 7675-95-8; **23**, 2232-16-8; **24**, 25062-45-7; **29**, 623-00-7; **30**, 25062-46-8; **32**, 25055-79-2; **38**, 3376-26-9.

(28) C. Kjellin and K. G. Kuglenstjerna, *Ber.*, **30** (1899).

(29) G. M. Badger, R. G. Buttery, and G. E. Lewis, *J. Chem. Soc.*, 2143 (1953).

(30) G. M. Badger and R. G. Buttery, *ibid.*, 2156 (1953).

(31) H. E. DeLaMare and G. M. Coppinger, *J. Org. Chem.*, **28**, 1068 (1963).

Carbodiimide–Sulfoxide Reactions. IX.[1] Synthesis of 2′- and 3′-Keto Derivatives of Cytidine

U. BRODBECK[2] AND J. G. MOFFATT*

Contribution No. 74 from the Institute of Molecular Biology, Syntex Research, Palo Alto, California 94304

Received April 1, 1970

Reaction of N^4-acetylcytidine with an excess of chlorotriphenylmethane in pyridine at 90° gives both the 2′,5- and 3′,5′-ditrityl derivatives and a small amount of N^4-acetyl-2′,3′,5′-tritritylcytidine. Each compound could be deacetylated and then related to the corresponding uridine derivatives by deamination. Efficient oxidation of the isomeric N^4-acetyl ditritylcytidines could be achieved using either the dimethyl sulfoxide–dicyclohexylcarbodiimide or the dimethyl sulfoxide–acetic anhydride methods giving the corresponding 2′- and 3′-ketocytidine derivatives. Subsequent detritylation using hydrogen chloride in chloroform gave N^4-acetyl-2′(or 3′)-ketocytidines. Oxidation of free 2′,5′- or 3′,5′-ditritylcytidines could also be accomplished using the DMSO–DCC method. Borohydride reduction of the various compounds was studied, the 2′ ketones giving nucleosides with the arabinose and ribose configurations in a ratio of 4:1 while the 3′ ketones gave xylosyl and ribosyl derivatives in a ratio of 3:2. Reduction of the free N^4-acetyl-2′- and -3′-ketocytidines with sodium borohydride-^{3}H provides a facile route to cytosine nucleosides with the arabinose, xylose, and ribose configurations containing a tritium label at specific positions of the sugar.

The development of mild methods for the oxidation of alcohols based upon the reactions of dimethyl sulfoxide (DMSO) activated by dicyclohexylcarbodiimide (DCC),[3] acetic anhydride,[4] or phosphorus pentoxide[5] has led to many syntheses of otherwise difficulty accessible keto sugar derivatives.[6] In an earlier paper in this series,[7] we have described the oxidation in good yield of 2′,5′-di-*O*-trityluridine (**1**) and of 3′,5′-di-*O*-

* Author to whom correspondence should be addressed.

(1) For part VIII, see A. H. Fenselau, E. H. Hamamura, and J. G. Moffatt, *J. Org. Chem.*, **35**, 3546 (1970).

(2) Syntex Postdoctoral Fellow, 1967–1968.

(3) K. E. Pfitzner and J. G. Moffatt, *J. Amer. Chem. Soc.*, **85**, 3027 (1963); 5661 (1965); **87**, 5670 (1965).

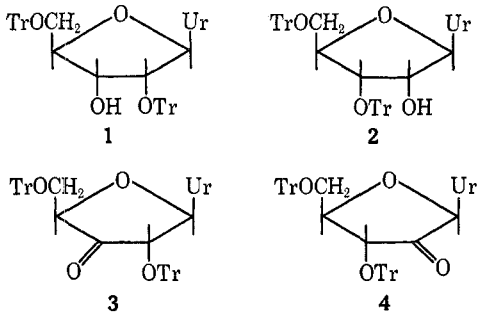
(4) J. D. Albright and L. Goldman, *ibid.*, **89**, 2416 (1967).

(5) K. Onodera, S. Hirano, and N. Kashimura, *Carbohyd. Res.*, **6**, 276 (1968).

(6) For reviews, see (a) J. S. Brimacombe, *Angew. Chem., Int. Ed. Engl.*, **8**, 401 (1969). (b) J. G. Moffatt in "New Oxidation Reactions," D. J. Trecker, Ed., Marcel Dekker, New York, N. Y., 1970, in press.

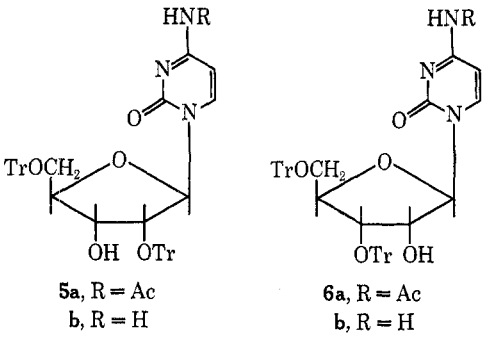
(7) A. F. Cook and J. G. Moffatt, *J. Amer. Chem. Soc.*, **89**, 2697 (1967).

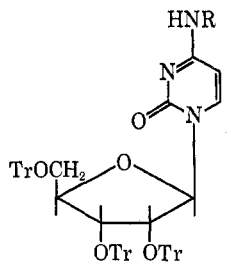
trityluridine (2) to the corresponding ditrityl ketonucleosides, **3** and **4**, respectively.



In this paper we describe an extension of this work to the synthesis of derivatives of cytidine and the use of these compounds as intermediates in facile syntheses of cytosine ribosides, arabinosides, and xylosides bearing isotopic labels in specific positions of the sugar.

In view of the great alkaline lability of 2′- and 3′-ketonucleosides,[7] the appropriate di-*O*-trityl derivatives of cytidine appear to be the most satisfactory starting materials for oxidation. The reaction of N^4-acetylcytidine[8] with 3 equiv of chlorotriphenylmethane in pyridine at 90° was found to give roughly equal amounts (33 and 25%) of N^4-acetyl-2′,5′-di-*O*-tritylcytidine (**5a**) and N^4-acetyl-3′,5′-di-*O*-tritylcytidine (**6a**), together with a small amount of N^4-acetyl-2′,3′-5′-tri-*O*-tritylcytidine (**7a**). The isomeric ditrityl compounds are readily distinguished by thin layer chromatography. Mizuno and Sasaki[9] have briefly described the tritylation of N^4-acetylcytidine but only reported the isolation of **5a**. Each compound was deacetylated by treatment with ammonium hydroxide giving crystalline 2′,5′-di-*O*-tritylcytidine (**5b**), 3′,5′-di-*O*-tritylcytidine (**6b**), and 2′,3′,5′-tri-*O*-tritylcytidine (**7b**), respectively. The structural assignments were confirmed by deamination of **5b**, **6b**, and **7b** with isoamyl nitrite and acetic acid



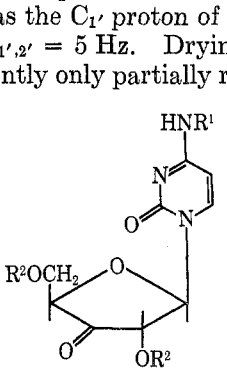


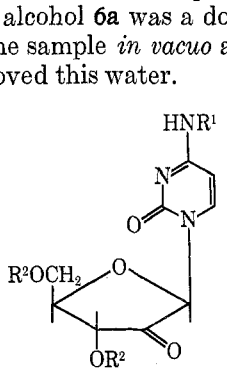
giving 2′,5′-di-*O*-trityluridine, 3′,5′-di-*O*-trityluridine, and 2′,3′,5′-tri-*O*-trityluridine all of which were identical with authentic samples[7] in their physical properties and spectroscopic behavior.

Oxidation of the *N*-acetyl nucleosides, **5a** and **6a**, was readily achieved using either the DMSO–acetic anhydride[4] or the DMSO–DCC[3] methods. Using the former method we have found it convenient to carry out the oxidation at 60° for 4 hr rather than at room temperature for an extended period. There was no observable formation of either acetate esters or thiomethoxy methyl ethers during oxidation of these compounds. Both methods of oxidation were very efficient, the reaction of **5a**, for example, giving the desired ketone, N^4-acetyl-1-(2′,5′-di-*O*-trityl-β-D-*erythro*-pentofuran-3-ulosyl)cytosine (**8a**),[10] in yields of 87 and 86%. The product was readily crystalline and the presence of the 3′-keto function was demonstrated by a carbonyl band at 1780 cm^{-1} in the infrared spectrum in addition to those normally present at roughly 1720 and 1675 cm^{-1} in the alcohol **5a**. The rather high frequency of the 3′-carbonyl group is similar to those previously observed for other ketofuranosides.[5,7]

Formation of the ketone also leads to striking alterations in the nmr spectra. Whereas in the alcohol **5a** all the sugar protons are well separated and resolved, the 1′, 2′, and 4′ protons of **8a** are superimposed as a signal at 4.7 ppm. In deuteriobenzene these three protons are resolved, both $C_{1'}H$ and $C_{2'}H$ appearing as singlets. The large shift of the anomeric proton from 6.75 ppm in **5a** to 4.7 ppm in **8a** is particularly striking.

In a similar way, the oxidation of N^4-acetyl-3′,5′-di-*O*-tritylcytidine (**6a**) to N^4-acetyl-1-(3′,5′-di-*O*-trityl-β-D-*erythro*-pentofuran-2-ulosyl)cytosine (**9a**)[10] was readily achieved using the DMSO–acetic anhydride method, the homogeneous product being obtained in 81% yield following preparative thin layer chromatography. Crystalline **9a** was obtained from ethanol as a hydrate and while covalent hydration of the 2′-keto function is a likely possibility, a new carbonyl band was present at 1780 cm^{-1} in its infrared spectrum. Whether or not the carbonyl group was covalently hydrated, the absence of a $C_{2'}$ proton was indicated by the appearance of the $C_{1'}$ proton as a singlet in the nmr spectrum whereas the $C_{1'}$ proton of the alcohol **6a** was a doublet with $J_{1',2'} = 5$ Hz. Drying the sample *in vacuo* at 80° apparently only partially removed this water.





The oxidation of the isomeric ditritylcytidines **5b** and **6b** containing free 4-amino functions was also accom-

(8) K. A. Watanabe and J. J. Fox, *Angew. Chem., Int. Ed. Engl.*, **5**, 579 (1966).

(9) Y. Mizuno and T. Sasaki, *Tetrahedron Lett.*, 4579 (1965).

(10) For simplicity we trivially refer to this compound (**8a**) as N^4-acetyl-3′-keto-2′,5′-di-*O*-tritylcytidine and use a similar nomenclature for related ketonucleosides elsewhere in this paper.

plished. In these cases, the use of the DMSO–acetic anhydride method was not feasible since concomitant acetylation of the amino function occurred, oxidation of **6b** giving the N^4-acetyl ketone (**9a**) in 76% yield. These oxidations were also attempted using DMSO and phosphorus pentoxide[5] at 60° and, while the desired products were formed, there were also considerable amounts of several by-products including detritylated materials. Oxidation of **5b** and **6b** could, however, be successfully achieved using the DMSO–DCC method. A number of model experiments indicated that dichloroacetic acid was perhaps the preferred proton source and that disappearance of the starting material was essentially complete using either 0.5 or 1.2 equiv of this acid. Examination of the reaction mixtures using tlc, however, showed that, in addition to the desired product (**8c** or **9c**), a major, much less polar, product was present to various extents in different experiments. Attempted isolation of this material led to partial breakdown to the desired ketone. The nonpolar product had a uv spectrum with λ_{max}^{MeOH} 307 mμ similar to an N^4-acetylcytidine derivative and its seems likely to be the N^4-dichloroacetyl derivative of the desired ketone. Brief treatment with very dilute methanolic ammonium hydroxide cleaved the acyl function without effecting the rest of the molecule and in this way crystalline **9c** was isolated in 71% yield.

Attempted hydrolysis of the trityl groups from the ditrityl ketones (**8a**, **8c**, **9a**, or **9c**) with acetic acid led to complete decomposition predominantly *via* glycosidic cleavage. On the other hand, treatment of **8a** or **9a** with roughly 3 mol equiv of anhydrous hydrogen chloride in chloroform at 0° led to precipitation of the free N^4-acetyl-3′- and -2′-ketocytidines (**8b** and **9b**) in good yields and contaminated with only traces of impurities. As was found in the uridine series, these compounds are unstable and tend to decompose upon prolonged storage or upon attempted purification by preparative tlc on cellulose. Neither compound was obtained in crystalline form and both undoubtedly exist as ketone hydrates since they do not show carbonyl absorptions other than those present in N^4-acetylcytidine in their ir spectra. As obtained, they were, however, essentially homogeneous by paper chromatography and electrophoresis, both having electrophoretic mobilities greater than that of N^4-acetylcytidine in pH 6.0 borate.[11] The structures of the compounds are clearly defined by the reduction experiments which follow. Attempted detritylation of the free amino ketones (**8c** and **9c**) using hydrogen chloride was unsuccessful owing to immediate formation of an insoluble hydrochloride.

As was shown in the uridine series,[7] both the 2′- and 3′-ketocytidine derivatives are very unstable under basic conditions, rapidly undergoing glycosidic cleavage. By a combination of spectral and chromatographic studies it could be shown that the ditrityl-2′- or 3′-ketones with or without an N^4-acetyl group were completely degraded within 10 min at room temperature in 0.01 *N* methanolic sodium hydroxide. The detritylated materials (**8b** and **9b**) were extremely unstable and decomposed instantly under comparable conditions.

(11) J. F. Codington, R. Fecher, and J. J. Fox, *J. Amer. Chem. Soc.*, **82**, 2794 (1960).

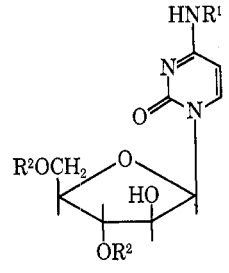
The optical rotatory dispersion (ORD) spectra of the various ketocytidine derivatives deserve some comment. Thus, all of the reported compounds (**8a–c**, **9a–c**) show positive Cotton effects as expected for pyrimidine β-nucleosides.[12] Also, the spectra of these compounds are roughly symmetrical and, in the case of the 2′-ketones (**9a–c**), show crossover at close to the λ_{max} of the ultraviolet spectra (*e.g.*, 390 mμ for **9a** and 255 mμ for **9c**). In the case of 3′-keto-2′,5′-di-*O*-tritylcytidine (**8c**), however, the spectrum is strongly shifted to longer wavelengths, crossover now occurring at 312 mμ which is roughly 50 mμ beyond the λ_{max} of 263 mμ. A somewhat similar, although less impressive, shift is found with **8a** which shows crossover at 319 mμ as compared with that of 300 mμ with the 2′-keto isomer (**9a**). These effects are not observed with **8b** and **9b** presumably because these compounds are known to be hydrates of the ketones. A similar effect was previously noted in the uridine series[7] where the ORD spectra of 3′-keto derivatives were markedly shifted toward longer wavelengths relative to their 2′-keto counterparts. It thus seems to be a general phenomenon that 3′-ketonucleosides exhibit Cotton effects in which the carbonyl group plays an important role relative to the heterocyclic base.

The borohydride reduction of the various ketones has been examined in some detail since, as will be seen, this provides a facile route to cytosine nucleosides labeled with tritium at specific positions of the sugar. The presence or absence of an N^4-acetyl group appears to have little effect upon the direction of reduction. Thus, reduction of either N^4-acetyl-2′-keto-3′,5′-ditritylcytidine or of the related 4-amino compound **9c** with sodium borohydride in ethanol–benzene gave products with arabinose and ribose configurations in a ratio of 4:1. These yields were determined by quantitative borate electrophoresis[11] following deacetylation with methanolic ammonium hydroxide and detritylation with acetic acid. Preparative reduction of **9a** showed that quite extensive loss of the N^4-acetyl group occurred concomitantly, but pure N^4-acetyl-1-(3′,5′-di-*O*-trityl-β-D-arabinofuranosyl)cytosine (**10a**) could be isolated by direct crystallization from the reaction mixture and shown to be pure by borate electrophoresis after removal of the protecting groups. If, subsequently, the deacetylation was completed by treatment of the reduction mixture with methanolic ammonium hydroxide, the expected 1-(3′,5′-di-*O*-trityl-β-D-arabinofuranosyl)cytosine (**10b**) and 3′,5′-di-*O*-tritylcytidine (**6b**) could be separated by preparative thin layer chromatography and isolated in crystalline form. Similar reduction of **9c** gave the same two compounds in isolated yields of 71 and 20%. The purity of each compound could be confirmed by detritylation followed by borate electrophoresis.

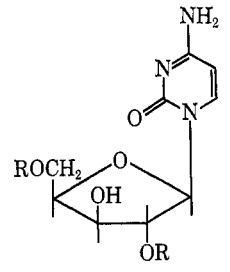
Reduction of the 3′-ketones (**8a** and **8c**) were also very similar and gave products with xylose and ribose configurations in a ratio of 3:2. Once again quite extensive deacetylation accompanied reduction of **8a**. In a preparative experiment, the deacetylation was completed by treatment with ammonium hydroxide prior to preparative thin layer chromatography which

(12) (a) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, **6**, 843 (1967); (b) T. Nishimura, B. Shimizu, and I. Iwai, *Biochim. Biophys. Acta*, **157**, 221 (1968).

clearly separated 1-(2′,5′-di-*O*-trityl-β-D-xylofuranosyl)-cytosine (**11a**) and 2′,5′-di-*O*-tritylcytidine (**5b**) which were isolated crystalline in yields of 55 and 37%.



10a, R^1 = Ac; R^2 = trityl
b, R^1 = H; R^2 = trityl
c, R^1 = R^2 = H



11a, R = trityl
b, R = H

For the preparation of nucleosides containing specific tritium labels in the sugar, it was considered desirable to minimize the number of chemical manipulations following introduction of the isotope. Accordingly, the reduction of the detritylated N^4-acetyl-2′- and -3′-ketocytidines (**9b** and **8b**) was examined. Treatment of **8b** or **9b** with sodium borohydride in water led to almost complete cleavage to cytosine presumably due to the extreme lability of these compounds under mildly alkaline conditions. In ethanol, however, little cytosine was formed and there was little indication of reduction of the cytosine ring.[13] Accordingly, **9b** was reacted in ethanol with a small excess of sodium borohydride-^{3}H and the crude product was then treated with ammonium hydroxide to complete removal of the N^4-acetyl group. A considerable amount of cytosine was formed and was most efficiently removed by preparative thin layer chromatography. The nucleoside band was further fractionated on a column of Dowex-1 (OH^-) resin according to Dekker[14] giving homogeneous cytidine-2′-^{3}H and 1-(β-D-arabinofuranosyl)cytosine-2′-^{3}H (**10c**) with specific activities of 0.58 and 0.54 mCi/μmol, respectively. Both compounds were homogeneous and identical with authentic samples in a variety of chromatographic and electrophoretic systems.

In a similar way the reduction of **8b** with sodium borohydride-^{3}H followed by deacetylation gave cytidine-3′-^{3}H and 1-(β-D-xylofuranosyl)cytosine-3′-^{3}H (**11b**).[15] In this case it was not necessary to remove cytosine by preparative thin layer chromatography and the two labeled nucleosides were clearly separated by chromatography on Dowex-1 (OH^-) resin. We were unable to obtain a satisfactory separation of these compounds using 30% methanol as eluent as recommended by Dekker[14] but got excellent separation using 20% methanol. Once again, the products were chromatographically and electrophoretically homogeneous. It should be pointed out that the total recovery of labeled nucleosides from reduction of **8b** and **9b** was only 26 and 40%. Since formation of cytosine was not a problem during reduction of these compounds with nonisotopic borohydride, it is not unlikely that some decomposition of the isotopic reagent had taken place prior to its use leading to some unreacted ketones which would decompose upon alkaline treatment. In retrospect, it is likely that reduction of the protected ketones **8c** or **9c** would provide better overall yields of the labeled nucleosides. In spite of these somewhat reduced yields obtained, this method provides a very direct and simple route for the preparation of biologically interesting nucleosides bearing specific isotopic labels.

(13) N. Miller and P. A. Cerutti, *J. Amer. Chem. Soc.*, **89**, 2767 (1967).
(14) C. A. Dekker, *ibid.*, **87**, 4028 (1965).
(15) We are grateful to Dr. J. J. Fox for an authentic sample of **11b**. J. J. Fox, N. Yung, I. Wempen, and I. L. Doerr, *ibid.*, **79**, 5060 (1957).

Experimental Section

General experimental methods are similar to those described previously.[1] Isotope counting was done using a Packard Tri-Carb liquid scintillation spectrometer using the scintillation fluid described by Herberg.[16]

Tritylation of N^4-Acetylcytidine.—N^4-Acetylcytidine (15 g, 52 mmol)[8] was added to a stirred solution of chlorotriphenylmethane (56.2 g, 157 mmol) in anhydrous pyridine (150 ml) at 90°. The clear solution that resulted after roughly 10 min was heated for a further 5 hr and then cooled and poured into 1.5 l. of well-stirred ice water. The resulting precipitate was dissolved in chloroform, extracted with 5% sodium bisulfate and water, dried ($MgSO_4$), and evaporated. The residue (70 g) was dissolved in benzene and applied to a 9 × 6 cm column of silicic acid which was then washed with benzene (2 l.) until no further triphenylmethanol (total 29.8 g) was eluted. Subsequent elution with chloroform–ethyl acetate (1:2) gave a mixture (30 g) of di- and tritrityl derivatives while elution with ethyl acetate gave a mixture (4 g) of unreacted N^4-acetylcytidine and monotrityl derivatives. The chloroform–ethyl acetate eluate was crystallized from chloroform–ether (using seed crystals previously obtained following preparative tlc) to give 9.54 g of pure N^4-acetyl-2′,5′-di-*O*-tritylcytidine (**5a**). The mother liquors were evaporated and the residue (19.6 g) was chromatographed on a column containing 700 g of silicic acid using chloroform–ethyl acetate (1:2). Following a small amount of triphenylmethanol, the first peak from the column was impure N^4-acetyl-2′,3′,5′-tri-*O*-tritylcytidine (**7a**) which was finally purified by preparative tlc using three consecutive developments with chloroform–ethyl acetate (2:1). The final product (1.2 g, 2%) was crystallized from benzene–ether with mp 265–266°: λ_{max}^{MeOH} 304 mμ (ε 5800), 250 (sh, 13,400); $[\alpha]^{23}D$ −10° (*c* 0.1, $CHCl_3$); ORD positive Cotton effect with peak at 324 mμ (Φ +15,600°), crossover at 305 mμ and a trough at 250 mμ (Φ −44,000°); nmr ($CDCl_3$) 2.27 ppm (s, 3, NAc), 2.3 (m, 1, $C_{5'a}H$), 2.71 (m, 1, J_{gem} = 11 Hz, $C_{5'b}H$), 3.45 (d, 1, $J_{2',3'}$ = 4.5 Hz, $C_{3'}H$), 4.0 (m, 1, $C_{4'}H$), 4.59 (q, 1, $J_{1',2'}$ = 8 Hz, $J_{2',3'}$ = 4.5 Hz, $C_{2'}H$), 6.53 (d, 1, $J_{5,6}$ = 8 Hz, C_5H), 6.8–7.5 (m, aromatic and $C_{1'}H$), 7.77 (d, 1, $J_{5,6}$ = 8 Hz, C_6H).

Anal. Calcd for $C_{68}H_{57}N_3O_6$: C, 80.67; H, 5.68; N, 4.15. Found: C, 80.76; H, 5.73; N, 4.31.

Continued elution then almost completely separated the ditrityl isomers **5a** and **6a**. The overlapping fractions were separated by preparative tlc using chloroform–ethyl acetate (1:2). Both isomers were then chromatographically homogeneous and distinct from one another. The faster isomer (**5a**, total yield 13.4 g, 33%) was readily recrystallized from ethanol or chloroform–hexane as fine needles which scintered and melted at 170–180° (reported[9] scintering and melting at 168–180°): λ_{max}^{MeOH} 305 mμ (ε 5400), 250 (sh, 13,100); $[\alpha]^{23}D$ +104° (*c* 0.1, $CHCl_3$); ORD (MeOH) positive Cotton effect with a peak at 330 mμ (Φ + 28,400°), crossover at 304 mμ and a trough at 250 mμ (Φ −41,000°); nmr ($CDCl_3$) 1.62 ppm (br s, 1, $C_{3'}OH$), 2.31 (s, 3, NAc), 2.84 (br d, 1, $J_{2',3'}$ = 5 Hz, $C_{3'}H$), 3.10 (q, 1, J_{gem} = 11 Hz, $J_{4',5'a}$ = 3 Hz, $C_{5'a}H$), 3.23 (q, 1, J_{gem} = 11 Hz, $J_{4',5'b}$ = 2 Hz, $C_{5'b}H$), 4.02 (br s, 1, $C_{4'}H$), 4.52 (q, 1, $J_{1',2'}$ = 7.5 Hz, $J_{2',3'}$ = 5 Hz, $C_{2'}H$), 6.75 (d, 1, $J_{1',2'}$ = 7.5 Hz, $C_{1'}H$), 6.97 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5H), 6.1–7.6 (m, 30, aromatic), 8.00 (d, 1, $J_{5,6}$ = 7.5 Hz, C_6H), 10.25 (br s, 1, NH).

Anal. Calcd for $C_{49}H_{43}N_3O_6$: C, 76.43; H, 5.63; N, 5.46. Found: C, 76.05; H, 5.76; N, 5.48.

The more polar isomer (**6a**, total yield 10.1 g, 25%) was a white solid which has not been obtained in crystalline form: λ_{max}^{MeOH} 300 mμ (ε 5700), 250 (sh, 13,600); $[\alpha]^{23}D$ +40° (*c* 0.1, $CHCl_3$); ORD positive Cotton effect with a peak at 318 mμ (Φ +11,300°), crossover at 304 mμ and a trough at 255 mμ (Φ −34,200°); nmr ($CDCl_3$) 1.88 ppm (br s, 1, $C_{2'}OH$), 2.27

(16) R. J. Herberg, *Anal. Chem.*, **32**, 43 (1960).

(s, 3, NAc), 2.90 (q, 1, J_{gem} = 11 Hz, $J_{4',5'a}$ = 3 Hz, $C_{5'a}H$), 3.33 (q, 1, J_{gem} = 11 Hz, $J_{4',5'b}$ = 2 Hz, $C_{5'b}H$), 3.60 (m, 1, $C_{4'}H$), 3.90 (t, 1, $J_{1',2'} = J_{2',3'}$ = 5 Hz, $C_{2'}H$, superimposed upon NH), 4.18 (q, 1, $J_{2',3'}$ = 5 Hz, $J_{3',4'}$ = 3 Hz, $C_{3'}H$), 6.06 (d, 1, $J_{1',2'}$ = 5 Hz, $C_{1'}H$), 7.0–7.6 (m, 30 aromatic and C_6H), 8.05 (d, 1, $J_{5,6}$ = 7.5 Hz, C_6H), 9.94 (br s, 1, NH).

Anal. Calcd for $C_{49}H_{43}N_3O_6$: C, 76.43; H, 5.63; N, 5.46. Found: C, 75.90; H, 5.74; N, 5.37.

2',5'-Di-*O*-tritylcytidine (5b).—Concentrated ammonium hydroxide (80 ml) was added to a solution of (**5a**) in chloroform (50 ml) and acetone (100 ml). Methanol was added until a clear solution resulted and this was stored overnight. Crystalline **5b** (4.11 g, 89%) was collected with mp 180–182° unchanged upon recrystallization from benzene–acetone: λ_{max}^{MeOH} 270 mμ (ε 8000), λ_{max}^{MeOH,H^+} 278 mμ (11,000); $[\alpha]^{23}D$ +117° (*c* 0.1, $CHCl_3$); ORD (MeOH) positive Cotton effect with peak at 292 mμ (Φ +35,400°), crossover at 272 mμ and a trough at 230 mμ (Φ −55,000°); nmr (DMSO-d_6) 2.5 ppm (m, 1, $C_{3'}H$), 2.9 (br s, 2, $C_{5'}H_2$), 3.80 (br s, 1, $C_{4'}H$), 4.15–4.35 (m, 2, $C_{2'}H$ and $C_{3'}OH$), 5.36 (d, 1, $J_{5,6}$ = 8 Hz, C_5H), 6.42 (d, 1, $J_{1',2'}$ = 7 Hz, $C_{1'}H$), 7.0–7.6 (m, 30 aromatic and C_6H).

Anal. Calcd for $C_{47}H_{41}N_3O_5$: C, 77.54; H, 5.68; N, 5.78. Found: C, 77.36; H, 5.67; N, 6.02.

Treatment of **5a** (100 mg) in DMSO (2 ml) and benzene (0.2 ml) with isoamyl nitrite (0.1 ml) and glacial acetic acid (0.05 ml) at 25° for 2 days followed by preparative tlc using chloroform–ethyl acetate (10:1) gave crystalline 2',5'-di-*O*-trityluridine (49 mg, 52%) that was chromatographically and physically identical with an authentic sample.[7] No 3',5'-di-*O*-trityluridine was formed.

3',5'-Di-*O*-tritylcytidine (6b).—Concentrated ammonium hydroxide (100 ml) was added to a solution of **6a** (5 g) in chloroform (10 ml) and methanol (200 ml), giving an initially clear solution. After 12 hr at 25°, crystalline **6b** (4.04 g, 87%) was removed and had mp 225–226° unchanged upon recrystallization from methanol: λ_{max}^{MeOH} 270 mμ (ε 8700), λ_{max}^{MeOH,H^+} 285 mμ (13,400); $[\alpha]^{23}D$ +48° (*c* 0.1, $CHCl_3$); ORD (MeOH) positive Cotton effect with a peak at 289 mμ (Φ +10,800°), crossover at 276 mμ and a trough at 236 mμ (Φ −29,000°); nmr ($CDCl_3$) 2.76 ppm (q, 1, J_{gem} = 11 Hz, $J_{4',5'a}$ = 4 Hz, $C_{5'a}H$), 3.22 (q, 1, J_{gem} = 11 Hz, $J_{4',5'b}$ = 2 Hz, $C_{5'b}H$), 3.43 (m, 1, $C_{4'}H$), 3.80 (t, 1, $J_{1',2'} = J_{2',3'}$ = 5 Hz, $C_{2'}H$), 4.12 (q, 1, $J_{2',3'}$ = 5 Hz, $J_{3',4'}$ = 3 Hz, $C_{3'}H$), 5.35 (*d*, 1, $J_{5,6}$ = 8 Hz, C_5H), 5.99 (d, 1, $J_{1',2'}$ = 5 Hz, $C_{1'}H$), 6.9–7.5 (m, 30, aromatic), 7.72 (d, 1, $J_{5,6}$ = 8 Hz, C_6H).

Anal. Calcd for $C_{47}H_{41}N_3O_5$: C, 77.54; H, 5.68; N, 5.78; Found: C, 77.36; H, 5.74; N, 5.92.

Treatment of **6b** (100 mg) with isoamyl nitrite and acetic acid as above gave 43 mg (46%) of chromatographically homogeneous 3',5'-di-*O*-trityluridine that was identical with an authentic sample by tlc and by infrared and nmr spectroscopy.

2',3',5'-Tri-*O*-tritylcytidine (7b).—Concentrated ammonium hydroxide (5 ml) was added to a solution of **7a** (400 mg) in methanol (50 ml) and the solution was heated under reflux for 2 hr. Preparative tlc using three developments with CCl_4–acetone (2:1) cleanly separated the product from unreacted **7a**. Elution and crystallization from methanol–water gave 70 mg of pure **7b** with mp 241–244°: λ_{max}^{MeOH} 277 mμ (ε 13,100); ORD (MeOH) positive Cotton effect with a peak at 302 mμ (Φ +32,500°) and crossover at 278 mμ; nmr ($CDCl_3$) 1.84 ppm (br s, 2, NH_2), 2.64 (d, 1, $J_{2',3'}$ = 5 Hz, $C_{3'}H$), 2.98 (br s, 2, $C_{5'}H_2$), 3.89 (d, 1, *J* = 1 Hz, $C_{4'}H$), 4.36 (q, 1, $J_{1',2'}$ = 7 Hz, $J_{2',3'}$ = 5 Hz, $C_{2'}H$), 4.39 (d, 1, $J_{5,6}$ = 8 Hz, C_5H), 6.74 (d, 1, $J_{1',2'}$ = 7 Hz, $C_{1'}H$), 6.8–7.6 (m, 45 aromatic and C_6H).

Anal. Calcd for $C_{66}H_{55}N_3O_5$: C, 81.70; H, 5.72; N, 4.33. Found: C, 81.77; H, 6.11; N, 4.63.

Treatment of **7b** (50 mg) with isoamyl nitrite (0.04 ml) and acetic acid (0.02 ml) in dioxane (2 ml) for 14 days led to roughly 50% deamination to 2',3',5'-tri-*O*-trityluridine which was isolated by preparative tlc using chloroform–ethyl acetate (10:1) and shown to be identical with an authentic sample.[7]

N^4-Acetyl-3'-keto-2',5'-di-*O*-tritylcytidine (8a). **A.**—A solution of **5a** (1.55 g, 2 mmol) and acetic anhydride (2 ml) in DMSO (20 ml) and anhydrous benzene (5 ml) was kept at 60° for 4 hr. The solution was then diluted with ethyl acetate, extracted once with 5% sodium bicarbonate and twice with water, dried ($MgSO_4$), and purified by preparative tlc on four 1-m long plates using carbon tetrachloride–acetone (2:1). Elution of the major band gave 1.37 g (87%) of chromatographically homogeneous **8a** which could be crystallized from methanol–acetone as needles which melted with decomposition at 196–198°, partially recrystallized and did not remelt below 300°: λ_{max}^{MeOH} 302 mμ (ε 6500), 250 (sh 16,600); $[\alpha]^{23}D$ +40° (*c* 0.1, $CHCl_3$); ORD (MeOH) positive Cotton effect with a peak at 331 mμ (Φ +10,800°), crossover at 319 mμ and a trough at 300 mμ (Φ −15,100°); ν_{max} (KBr) 1780, 1730, 1660 cm^{-1}; nmr ($CDCl_3$) 1.84 ppm (s, 3, NAc), 3.4 (m, 2, $C_{5'}H_2$), 4.5–4.8 (m, 3, $C_{1'}H$, $C_{2'}H$, $C_{4'}H$), 6.45 (d, 1, $J_{5,6}$ = 8 Hz, C_5H), 6.5–7.5 (m, 31, aromatic and C_6H), 10.12 (s, 1, NH).

Anal. Calcd for $C_{49}H_{41}N_3O_6$: C, 76.63; H, 5.38; N, 5.48. Found: C, 76.54; H, 5.34; N, 5.63.

B.—Dichloroacetic acid (0.02 ml, 0.25 mmol) was added to a solution of **5a** (385 mg, 0.5 mmol) and DCC (309 mg, 1.5 mmol) in a mixture of benzene (5 ml) and DMSO (5 ml) and kept overnight at 25°. The mixture was diluted with ethyl acetate and a solution of oxalic acid (1.5 mmol) in methanol (0.5 ml) was added. After 30 min the solution was filtered, extracted with 5% sodium bicarbonate and then twice with water, dried ($MgSO_4$), and evaporated to dryness. The residue was dissolved in acetone (5 ml), filtered to remove a small amount of dicyclohexylurea, and evaporated. Crystallization of the residue from ether gave 330 mg (86%) of pure **8a** identical with that above.

3'-Keto-N^4-acetylcytidine (8b).—An anhydrous solution of hydrogen chloride in chloroform (6.2 ml of 0.27 *N*, 1.7 mmol) was added to a solution of **8a** (389 mg, 0.5 mmol) in chloroform (10 ml) and kept for 1 hr at 0°. After addition of ether (30 ml) the white precipitate was collected by centrifugation in a tube protected by a serum cap and washed three times with fresh ether. After drying *in vacuo* over phosphorus pentoxide and potassium hydroxide pellets **8b** (160 mg) was obtained as a very hygroscopic white powder which moved as a single spot just faster than N^4-acetylcytidine and gave a positive test with dinitrophenylhydrazine spray on borate electrophoresis (1 *M* boric acid, pH 6.0).[11] It also gave an intense spot upon paper chromatography using 1-butanol–H_2O (86:14) with only traces of impurities present: λ_{max}^{MeOH} 297 mμ (ε 7100), 248 (13,400), 213 (16,200); λ_{max}^{MeOH,H^+} 314 mμ (ε 13,400), 230 (sh, 8100) and 214 (12,100); $[\alpha]^{23}D$ +81° (*c* 0.1, $CHCl_3$); ORD (MeOH) positive Cotton effect with a peak at 315 mμ (Φ +9300°), crossover at 290 mμ and a trough at 250 mμ (Φ −16,000°); ν_{max} (KBr) 1715, 1600 cm^{-1}; nmr (DMSO-d_6) 2.13 ppm (s, 3, NAc), 3.68 (br d, 2, $J_{4',5'}$ = 3 Hz, $C_{5'}H_2$), 4.3 (m, 1, $C_{4'}H$), 4.34 (d, 1, $J_{1',2'}$ = 7.5 Hz, $C_{2'}H$), 6.14 (d, 1, $J_{1',2'}$ = 7.5 Hz, $C_{1'}H$), 7.26 (d, 1, $J_{5,6}$ = 8 Hz, C_5H), 8.35 (d, 1, $J_{5,6}$ = 8 Hz, C_6H).

Anal. Calcd for $C_{11}H_{13}N_3O_6 \cdot 2H_2O$: C, 41.36; H, 5.37; N, 13.15. Found: C, 40.99; H, 5.41; N, 12.49.

2'-Keto-3',5'-di-*O*-trityl-N^4-acetylcytidine (9a). **A.**—Acetic anhydride (2 ml) was added to a solution of **6a** (1.54 g, 2 mmol) in DMSO (19 ml) and kept at 60° for 2 hr. The reaction was worked up as for **8a** and purified by preparative tlc on four plates using carbon tetrachloride–acetone (2:1). Elution of the major ultraviolet absorbing band gave 1.24 g (81%) of homogeneous **9b** as a granular foam that could be crystallized from ethanol and melted with decomposition at 158–160°: λ_{max}^{MeOH} 300 mμ (ε 6000), 249 (15,600); λ_{max}^{MeOH,H^+} 316 mμ (ε 14,900); $[\alpha]^{23}D$ +37° (*c* 0.1, $CHCl_3$); ORD positive Cotton effect with a peak at 315 mμ (Φ +4400°), crossover at 300 mμ and at rough at 256 mμ (Φ −21,700°); ν_{max} (KBr) 1780, 1730, 1660 cm^{-1}; nmr ($CDCl_3$) 2.06 ppm (s, 3, NAc), 2.72 (m, 1, $C_{5'a}H$), 3.02 (m, 1, $C_{5'b}H$), 4.15 (m, 1, $C_{4'}H$), 4.45 (d, 1, $J_{3',4'}$ = 5 Hz, $C_{3'}H$), 5.60 (s, 1, $C_{1'}H$), 7.0–7.5 (m, 31, aromatic and C_5H), 7.58 (d, 1, $J_{5,6}$ = 8 Hz, C_6H).

Anal. Calcd for $C_{49}H_{41}N_3O_6 \cdot H_2O$: C, 74.88; H, 5.51; N, 5.35. Found: C, 74.95; H, 5.40; N, 5.35.

B.—A solution of **6b** (500 mg) and acetic anhydride (0.5 ml) in DMSO (10 ml) was kept at 60° for 4 hr. The mixture was then partitioned between ethyl acetate and water and the organic phase was extracted with cold aqueous sodium bicarbonate and then water. It was then purified by preparative tlc using carbon tetrachloride–acetone (3:1) and the major band was eluted and crystallized from acetone–methanol giving 380 mg (76%) of essentially pure **9a** with mp 155–160° dec.

2'-Keto-N^4-acetylcytidine (9b).—A solution of **9a** (1.49 g, 1.94 mmol) in chloroform (5 ml) was cooled to 0° and a solution of anhydrous hydrogen chloride in chloroform (20 ml of 0.41 *N*, 8.2 mmol) was added in four portions over 15 min. After 1 hr at 0° the mixture was diluted with ether (50 ml) and the white precipitate was collected by centrifugation. It was then washed three times with ether and dried *in vacuo* over potassium hy-

droxide giving 0.46 g of **9b** as a hygroscopic powder which gave a single, dinitrophenylhydrazine positive spot similar to **8b** on borate electrophoresis and paper chromatography: λ_{max}^{MeOH} 299 mμ (ε 6700), 248 (13,200), 214 (15,300); λ_{max}^{MeOH,H^+} 315 mμ (ε 14,400), 230 (sh, 8200), 215 (9900); $[\alpha]^{23}D$ +95°; ORD (MeOH) positive Cotton effect with a peak at 318 mμ (Φ +8900°), crossover at 295 mμ and a trough at 250 mμ (Φ −21,200°); ν_{max} (KBr) 1720, 1600 cm^{-1}; nmr (DMSO-d_6) 2.13 ppm (s, 3, NAc), 3.5–4.0 (m, 5, C$_4$'H, C$_5$'H$_2$, C$_3$'OH and C$_5$'OH), 4.41 (d, 1, $J_{3',4'}$ = 8 Hz, C$_3$'H), 5.48 (s, 1, C$_1$'H), 7.24 (d, 1, $J_{5,6}$ = 8 Hz, C$_5$H), 4.19 (d, 1, $J_{5,6}$ = 8 Hz, C$_6$H). Elemental analysis indicated that the sample was roughly a dihydrate but acceptable figures were not obtained for all elements. The ultraviolet and ORD values quoted above are based upon this hydrate.

3'-Keto-2',5'-di-*O*-tritylcytidine (8c).—Dichloroacetic acid (0.1 ml, 1.2 mol) was added to a solution of **5b** (727 mg, 1 mmol) and DCC (618 mg, 3 mmol) in benzene (5 ml) and DMSO (20 ml) and the mixture was stored overnight. The mixture was worked up as above for **8a** (method B) and purified by preparative tlc using carbon tetrachloride–acetone (2:1) giving two bands, one moving somewhat faster than the starting material and the other near the solvent front. Elution of the slower band gave 370 mg (51%) of chromatographically homogeneous **8c** which could be crystallized from methanol with mp 142–144°: λ_{max}^{MeOH} 263 mμ (sh, ε 8600), λ_{max}^{MeOH,H^+} 285 (11,300); $[\alpha]^{23}D$ +35° (*c* 0.1, $CHCl_3$); ORD (MeOH) positive Cotton effect with a peak at 332 mμ (Φ +9800°), crossover at 312 and a trough at 286 mμ (Φ −13,700°); nmr (DMSO-d_6) 3.3 (m, 2, C$_5$'H$_2$), 4.5 (m, 2, C$_2$'H and C$_4$'H), 4.95 (d, 1, $J_{1',2'}$ = 2.5 Hz, C$_1$'H), 5.49 (d, 1, $J_{5,6}$ = 7 Hz, C$_5$H), 6.66 (d, 1, $J_{5,6}$ = 7 Hz, C$_6$H); 7.05 (br s, 2, NH$_2$), 7.1–7.4 (m, 30, aromatic).

Anal. Calcd for $C_{47}H_{39}N_3O_5$: C, 77.76; H, 5.42; N, 5.79. Found: C, 77.55; H, 5.57; N, 5.76.

Elution of the fast band gave 390 mg of a material that had already partially decomposed to **8c**. Preparative tlc using chloroform–ether (10:1) separated some crystalline 1-dichloroacetyl-1,3-dicyclohexylurea of mp 147–148° (lit.[17] mp 146–148°) but was accompanied by extensive decomposition of the fast band to **8c**. Elution and crystallization of the resulting band on the origin gave a further 140 mg (total yield 70%) of **8c** identical with that above.

2'-Keto-3',5'-di-*O*-tritylcytidine (9c).—Dichloroacetic acid (0.1 ml, 1.2 mmol) was added to a solution of **6b** (727 mg, 1 mmol) and DCC (618 mg, 3 mmol) in a mixture of benzene (10 ml) and DMSO (10 ml). After storage overnight, the reaction was worked up as described for **8a** (method B). After the oxalic acid treatment and aqueous extraction tlc using chloroform–methanol (10:1) showed the presence of **9c** and a fast-moving compound in a ratio of roughly 1:3. The solvent was evaporated and the residue dissolved in methanol (10 ml). Concentrated ammonium hydroxide (0.4 ml) was added and after 5 min the solvent was rapidly removed and the residue was purified by preparative tlc using chloroform–methanol (10:1). Elution of the major band gave 0.62 g of homogeneous **9c** that was crystallized from acetone–methanol giving 514 mg (71%) of needles with mp 153–154°: λ_{max}^{MeOH} 268 mμ (sh, 8400), λ_{max}^{MeOH,H^+} 283 mμ (13,100); $[\alpha]^{23}D$ +75° (*c* 0.1, $CHCl_3$); ORD (MeOH) positive Cotton effect with a peak at 273 mμ (Φ +10,700°), crossover at 255 and a trough at 238 mμ (Φ −4400°); ν_{max} (KBr) 1780, 1650 cm^{-1}; nmr (DMSO-d_6) 5.47 ppm (s, 1, C$_1$'H), 5.79 (d, 1, $J_{5,6}$ = 7 Hz, C$_5$H), 7.0–7.4 (m, aromatic), 7.72 (d, 1, $J_{5,6}$ = 7 Hz, C$_6$H).

Anal. Calcd for $C_{47}H_{39}N_3O_5$: C, 77.76; H, 5.42; N, 5.79. Found: C, 77.70; H, 5.21; N, 6.36.

Reduction of 3'-Keto-2',5'-di-*O*-trityl-N^4-acetylcytidine (8a).—A solution of **8a** (200 mg, 0.26 mmol) in benzene (2 ml) was diluted with ethanol (10 ml) and sodium borohydride (5 mg, 0.13 mmol) was added. After storage in the dark for 20 min, the solvent was evaporated and the residue partitioned between chloroform and water. The chloroform phase was evaporated to dryness and the residue dissolved in a mixture of chloroform (5 ml) and methanol (10 ml). Concentrated ammonium hydroxide (2 ml) was added and after 3 hr at 20°, the solvent was evaporated and the residue purified by preparative tlc using carbon tetrachloride–acetone (1:1). Two bands resulted and elution of the slower one (74 mg) followed by crystallization from ethanol gave 70 mg (37%) of 2',5'-di-*O*-tritylcytidine (**5b**) that was identical to an authentic sample. Elution of the faster band (120 mg) followed by crystallization from ethanol gave 104 mg (55%) of 1-(2',5'-di-*O*-trityl-β-D-xylofuranosyl)cytosine (**11a**) which melted at 178–180°, resolidified, and remelted at roughly 250° (dec): λ_{max}^{MeOH} 267 mμ (ε 7700), λ_{max}^{MeOH,H^+} 288 mμ (ε 11,400); $[\alpha]^{23}D$ +37° (*c* 0.28, $CHCl_3$); ORD (MeOH) positive Cotton effect with a peak at 290 mμ (Φ +11,600°), crossover at 272 mμ and a trough at 250 mμ (Φ − 8200°); nmr (DMSO-d_6) 2.79 ppm (m, 2, C$_5$'H$_2$), 5.00 (d, 1, $J_{H,OH}$ = 4 Hz, C$_3$'OH), 5.68 (d, 1, $J_{5,6}$ = 7.5 Hz, C$_5$H), 6.25 (s, 1, C$_1$'H), 7.2–7.5 (m, 30, aromatic), 7.57 (d, 1, $J_{5,6}$ = 7.5 Hz, C$_6$H).

Anal. Calcd for $C_{47}H_{41}N_3O_5$: C, 77.56; H, 5.68; N, 5.77. Found:[18] C, 76.92; H, 5.68; N, 5.76.

Reduction of 2'-Keto-3',5'-di-*O*-tritylcytidine (9c).—Sodium borohydride (6 mg, 0.16 mmol) was added to a solution of **9c** (200 mg, 0.28 mmol) in a mixture of benzene (2 ml) and ethanol (10 ml). After 20 min the solvent was evaporated and the residue partitioned between chloroform and water. The organic phase was dried and separated into two bands by preparative tlc using chloroform–2-propanol (9:1). Elution of the faster band gave 41 mg (20%) of 3',5'-di-*O*-tritylcytidine that was identical with an authentic sample after crystallization from methanol. Elution of the slower band gave 143 mg (71%) of 1-(3',5'-di-*O*-trityl-β-D-arabinofuranosyl)cytosine (**10b**) which was crystallized from acetone as needles of mp 265–266°: λ_{max}^{MeOH} 271 mμ (ε 9400); $[\alpha]^{23}D$ +110° (*c* 0.23, $CHCl_3$); ORD (MeOH) positive Cotton effect with a peak at 288 mμ (Φ +14,600°), crossover at 272 mμ, and a trough at 235 mμ (Φ −30,900°); nmr ($CDCl_3$) 5.30 ppm (d, 1, $J_{5,6}$ = 7.5 Hz, C$_5$H), 6.18 (d, 1, $J_{1',2'}$ = 2 Hz, C$_1$'H), 7.57 (d, 1, $J_{5,6}$ = 7.5 Hz, C$_6$H).

Anal. Calcd for $C_{47}H_{41}N_3O_5$: C, 77.56; H, 5.68; N, 5.77. Found: C, 77.78; H, 5.18; N, 6.27.

N^4-Acetyl-1-(3',5'-di-*O*-trityl-β-D-arabinofuranosyl)cytosine (10a).—A solution of **9a** (150 mg, 0.2 mmol) in benzene (2 ml) and ethanol (15 ml) was treated with sodium borohydride (4 mg) for 20 min. After work-up as above, tlc showed that quite extensive deacetylation had occurred. Direct crystallization of the chloroform extracts from ethanol gave 23 mg of the pure N^4-acetyl arabinoside **10a** with mp 272–274°: λ_{max}^{MeOH} 300 (ε 8500), 245 mμ (sh, 16,000); λ_{max}^{MeOH,H^+} 313 mμ (14,200); ORD (MeOH) positive Cotton effect with a peak at 316 mμ (Φ +5500°), crossover at 302 mμ and a trough at 250 mμ (Φ −39,000°).

Anal. Calcd for $C_{49}H_{43}N_3O_6$: C, 76.43; H, 5.63; N, 5.46. Found: C, 76.07; H, 5.95; N, 5.38.

Deacetylation with ammonium hydroxide–methanol followed by detritylation with 80% acetic acid gave only arabinosylcytosine as judged by borate electrophoresis.

A comparable reduction of **9a** (100 mg) in which deacetylation was completed by treatment with concentrated ammonium hydroxide in methanol prior to preparative tlc as in the reduction of **8a** gave 67 mg (70%) of **10b** and a small amount of **6b**.

Reduction of 2'-Keto-N^4-acetylcytidine with $NaBH_4$-^3H.—A solution of sodium borohydride-^3H (50 mCi, specific activity 2.3 mCi/μmol) in ethanol (0.5 ml) was added to a solution of 2'-keto-N^4-acetylcytidine (13.7 mg, 36 μmol) in ethanol (1 ml) and the mixture was kept at room temperature for 4 hr. A little Dowex 50 (H^+) resin was then added, the mixture was filtered, and the resin was washed with 1 *N* ammonium hydroxide. The filtrates were evaporated and the residue was deacetylated by overnight storage in methanol (1 ml) and concentrated ammonium hydroxide (1 ml). After evaporation of the solvent, the residue was chromatographed on two 20 × 20 cm preparative tlc plates using 2-propanol–ethyl acetate–water (23:65:12) to separate the labeled nucleosides from cytosine. The radioactive region was eluted with methanol, evaporated, and applied to a 1 × 20 cm column of Dowex 1 (OH^-) resin equilibrated with methanol–water (3:7). Elution with the same solvent gave a symmetrical peak containing 3.0 μmol (8.4%) of cytidine-2'-^3H with a specific activity of 0.58 mCi/μmol. Continued elution with a linear gradient (0–0.1 *M*) of triethylammonium bicarbonate in 30% methanol (2 l.) then gave a second peak containing 11.5 μmol (32%) of 1-(β-D-arabinofuranosyl)cytosine-2'-^3H with a specific activity of 0.54 mCi/μmole. The pooled peaks were evaporated to dryness and then coevaporated several times with methanol to remove residual bicarbonate. Both peaks were homogeneous and identical with authentic standards

(17) M. G. Burdon and J. G. Moffatt, *J. Amer. Chem. Soc.*, **88**, 5855 (1966).

(18) In several preparations of this compound we have consistently obtained low carbon analyses.

by borate electrophoresis at pH 6, electrophoresis in 1 *M* acetic acid, and by paper chromatography in 1-butanol–acetic acid–water (4:1:1).

Reduction of 3′-Keto-N^4-acetylcytidine with $NaBH_4$-^3H.—A reaction between 3′-keto-N^4-acetylcytidine (17.3 mg, 41 μmol) and sodium borohydride-^3H (50 mCi, 2.3 mCi/μmol) was carried out as with the 2′ ketone above. After deacetylation, the mixture was evaporated to dryness and directly applied to a 2 × 45 cm column of Dowex-1 (OH^-) resin equilibrated with methanol–water (1:4). Continued elution with the same solvent gave two well resolved peaks centered about fractions 230 and 290 (15 ml each). The first of these contained cytidine-3′-^3H (3.5 μmol, 8.5%) with a specific activity of 0.62 mCi/μmol, while the second contained 7 μmol (17.1%) of 1-(β-D-xylofuranosyl)cytosine-3′-^3H with a specific activity of 0.54 mCi/μmol. Both products were homogeneous and identical with authentic samples by the electrophoretic and chromatographic systems above.

Registry No.—**5a**, 6698-19-7; **5b**, 6614-56-8; **6a**, 25787-17-1; **6b**, 25767-18-2; **7a**, 25787-19-3; **7b**, 25787-20-6; **8a**, 25787-21-7; **8b**, 25787-22-8; **8c**, 25787-23-9; **9a**, 25787-24-0; **9b**, 25787-25-1; **9c**, 25787-26-2; **10a**, 25787-27-3; **10b**, 25787-28-4; **11a**, 25834-65-5.

Notes

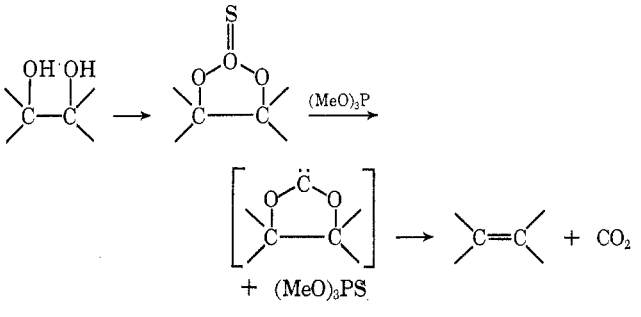
Evidence for a Carbenoid Intermediate in the Corey–Winter Alkene Synthesis[1,2]

DEREK HORTON AND CHARLES G. TINDALL, JR.

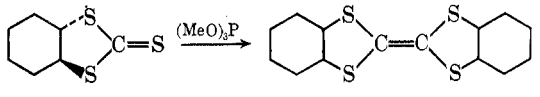
Department of Chemistry, The Ohio State University, Columbus, Ohio 43210

Received March 16, 1970

The synthesis of alkenes by treatment of cyclic 1,2-thionocarbonates with a phosphite ester (Corey–Winter alkene synthesis[3]) has proved to be a useful route to unsaturated sugar derivatives.[4–7] It was postulated[3] that the reaction proceeds through a carbene intermediate that is unstable with respect to the alkene and carbon dioxide.



Corey and coworkers[8] extended this synthesis to the preparation of alkenes from trithiocarbonates. The synthetic route permits preparation of highly strained alkenes in good yield. When formation of an alkene is impossible, as with *trans*-cyclohexane-1,2-dithiol 1,2-thionocarbonate, coupling products are obtained. These observations led the authors[8] to propose a



concerted, cycloelimination mechanism for the product-forming step. More recently Corey and Märkl[9] have isolated phosphorus ylides from the reaction of cyclic 1,3-trithiocarbonates with alkyl phosphites, and have been able to inhibit alkene formation from the cyclic 1,2-trithiocarbonates by adding an excess of benzaldehyde to the reaction mixture. Under the latter conditions the product is a ketene dithioacetal formed by a Wittig reaction of the intermediate ylide with the aldehyde. Some systems led only to alkenes, even when an excess of aldehyde was present. It was postulated[9] that ylide intermediates were formed in each case, at least with the trithiocarbonate precursors, and that competitive decomposition of the ylide to alkene, or reaction of the ylide with aldehyde, determined the product obtained.

We now present direct evidence to support the hypothesis of a carbene intermediate in the conversion of the thionocarbonate of a 1,2-diol into an alkene. The 5,6-thionocarbonate (**1**) of 1,2-*O*-isopropylidene-α-D-glucofuranose when treated with refluxing trimethyl phosphite for 70 hr gave, in addition to the 5,6-alkene **2** (isolated crystalline in 75% yield) as earlier reported,[5] a second product (**3**), isolated crystalline in 1% yield after column chromatography of the mother liquors from crystallization of **2**. Compound **3** proved to be identical in all respects with 1,2-*O*-isopropylidene-α-D-glucofuranose 3,5,6-orthoformate,[10] an authentic sample of which was prepared in 94% yield by condensation of 1,2-*O*-isopropylidene-α-D-glucofuranose with triethyl orthoformate.

(1) Part XI in the series Synthesis and Reactions of Unsaturated Sugars. For part X, see D. M. Clode, D. Horton, M. H. Meshreki, and H. Shoji, *Chem. Commun.*, 693 (1969).

(2) Supported, in part, by the Agricultural Research Service, U. S. Department of Agriculture, Grant No. 12-14-100-9201(71) (OSURF Project 2573) administered by the Northern Utilization Research and Development Division, Peoria, Ill.

(3) E. J. Corey and R. A. E. Winter, *J. Amer. Chem. Soc.*, **85**, 2677 (1963).

(4) D. Horton and W. N. Turner, *Tetrahedron Lett.*, 2531 (1964).

(5) D. Horton and W. N. Turner, *Carbohyd. Res.*, **1**, 444 (1966); D. Horton, J. K. Thomson, and C. G. Tindall, Jr., *Methods Carbohyd. Chem.*, **6**, in press.

(6) E. L. Albano, D. Horton, and T. Tsuchiya, *Carbohyd. Res.*, **2**, 349 (1966).

(7) D. Horton and C. G. Tindall, Jr., *Abstr. Pap. Amer. Chem. Soc. Meeting*, **158**, CARB6 (1969).

(8) E. J. Corey, F. A. Carey, and R. A. E. Winter, *J. Amer. Chem. Soc.*, **87**, 934 (1965).

(9) E. J. Corey and G. Märkl, *Tetrahedron Lett.*, 3201 (1967).

(10) K. Freudenberg and W. Jacob, *Ber.*, **80**, 325 (1947); E. J. Hedgley and O. Mérész, *Proc. Chem. Soc.*, 399 (1964).