Facile Addition of Hydroxylic Nucleophiles to the Formyl Group of Uridine-6-carboxaldehydes†

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The "instability" previously ascribed to the tri-O-protected 6-formyluridines has been shown to be due to a facile hydration of the aldehyde functionality. Using an efficient protecting group strategy, 2′,3′,5′-tris-*O*-(methoxymethyl)uridine-6-carboxaldehyde (**2**) was prepared in two steps from uridine. Aldehyde **2** was shown to undergo a reversible hydration reaction in $(CD_3)_2SO$ solution to give the *gem*-diol **3**, according to extensive ^1H and ^{13}C 1D and 2D NMR spectroscopic analyses. This *gem*-diol was the sole structure detected in D_2O solution. The diethyl acetal derivative **4** provided spectral data for comparison to that of **3**. Compound **2** afforded diastereomeric hemiacetal products upon treatment with alcohols, and deprotection of **2** afforded the heretofore unknown uridine-6-carboxaldehyde, which was found to exist as a 1:10 mixture of free aldehyde A and a 5′-cyclic hemiacetal structure D in dry $(CD_3)_2SO$ solution, but as a 1:2 mixture of a 5′-cyclic hemiacetal structure D and the *gem*-diol B in D_2O solution, by NMR.

Introduction

In 1972, Klein and Fox reported the preparation of tri-*O*-acetyl-6-formyluridine ("tri-*O*-acetylorotidine aldehyde") and stated that this compound was "unstable and, even in chloroform, it decomposed on standing".[1] Since this first report, others have prepared 6-formyl-2′,3′-*O*-isopropylidene-5′-*O*-trityluridine[2] and 6-formyl-2′,3′-*O*-isopropylidene-5′-*O*-(methoxymethyl)uridine,[3] but these investigators did not attempt to purify or study their formyluridines and instead, noting that this class of compounds was known to be unstable, they promptly derivatized the aldehyde moiety. We were interested in examining the properties of 6-formyluridine derivatives to analyze the exact nature of their unusually high chemical reactivity[4] and further wished to determine the effect that this reactivity might have on the structure of the unprotected parent compound. Herein, we report direct evidence that the "instability" which has been ascribed to this class of compounds is in fact due to a remarkably facile hydration phenomenon. In addition, we report the first preparation of a deblocked 6-formyluridine and describe this compound's unusual proclivity to forming cyclonucleoside structures in aqueous and nonaqueous solution.

Results and Discussion

Rather than relying upon the standard two-step nucleoside protection methodology of 2′,3′-*O*-isopropylidenation followed by separate 5′-hydroxyl group protection, we began our investigation by seeking a base-stable protecting group which could be easily attached to each and every ribofuranosyl hydroxyl group in one step. We discovered that uridine could be rapidly tri-*O*-protected with methoxymethyl groups within 4 h at 25 °C in 1:1 $(CH_3O)_2CH_2/CH_2Cl_2$ containing a catalytic amount of CF_3SO_3H (65%).[5] As shown in Scheme I, the protected nucleoside **1** thus obtained was equilibrated with 3 equiv of LDA in dry THF solution at −78 °C for 1 h according to uridine dianion methodology developed by Miyasaka's group,[3] and the resulting dianion was quenched with an excess of ethyl formate to afford, after acidic workup and purification by radial chromatography, 2′,3′,5′-tris-*O*-(methoxymethyl)uridine-6-carboxaldehyde (**2**, 50% yield, 73% based upon unrecovered starting material). We were unable ever to effect a more complete conversion of **1** to **2**, even with additional LDA, and suspect that the abundance of LDA-chelating ether oxygen atoms in our starting material prevented complete dianion formation from occurring.

The protected 6-formyluridine **2** was found to undergo a remarkably facile hydrate formation to afford the corresponding *gem*-diol **3** in $(CD_3)_2SO$ solution at room temperature when treated with 1–12 equiv of D_2O, by ^1H and ^{13}C NMR spectral analysis. The rate of hydrate formation was found to be slow ($t_{1/2}$ ~2 h) in samples free of acidic impurities, but the establishment of an equilibrium mixture of **2** and **3** was essentially instantaneous (by NMR) when such propitious impurities apparently were present or when a trace amount of HCl(g) was intentionally added. Similar hydration reactions were observed using H_2O in place of D_2O and using $CDCl_3$ in place of $(CD_3)_2SO$ as solvent, although experiments employing the latter substitution were limited by the low solubility of water. As shown in Figures 1 and 2, respectively, a discrete set of proton and carbon signals were observed in these NMR spectra for the aldehyde and its hydrate; thus, the rate of interconversion of these species under the conditions employed is slower than the NMR time scale (ν^{-1} ~10^{-8} s). An equilibrium constant K_{eq} for the formation of hydrate of 1.3 M^{-1} was determined from an integration of several intermediate ^1H NMR spectra. The structure determination of hydrate **3** was based in part upon its ^1H, ^{13}C, COSY, and short- and long-range ^1H–^{13}C HETCOR NMR spectral features, which compared favorably to those of the diethyl acetal **4** prepared from **2** in $(EtO)_3CH$ containing CF_3SO_3H as catalyst (89% yield). For example, the H5 proton resonance of **3** was found at δ 5.94, whereas that of **4** occurred at δ 5.84. The C5 carbon resonance of **3** was at δ 100.6, while that of **4** was at δ 102.2. Hydrate **3** prepared by the addition of H_2O to **2** revealed a diag-

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(1) Klein, R. S.; Fox, J. J. *J. Org. Chem.* **1972**, *37*, 4381.
(2) Rosenthal, A.; Dodd, R. H. *Carbohydr. Res.* **1980**, *85*, 15.
(3) (a) Tanaka, H.; Nasu, I.; Miyasaka, T. *Tetrahedron Lett.* **1979**, 4755. (b) Tanaka, H.; Hayakawa, H.; Miyasaka, T. *Chem. Pharm. Bull.* **1981**, *29*, 3565. (c) Tanaka, H.; Hayakawa, H.; Miyasaka, T. *Tetrahedron* **1982**, *38*, 2635.
(4) In an unrelated study, 1,3-dimethyl-6-formyluracil was shown to undergo spontaneous hydrate and hemiacetal formation: Silverman, R. B.; Groziak, M. P. *J. Am. Chem. Soc.* **1982**, *104*, 6434. Also, hydration of the formyl group of uracil-6-carboxaldehyde was documented in ref 10 of Otter, B. A.; Falco, E. A.; Fox, J. J. *J. Org. Chem.* **1978**, *43*, 481.
(5) A variation of the P_2O_5 method described in: Fuji, K.; Nakano, S.; Fujita, E. *Synthesis* **1975**, 276. To our knowledge, ours is the first report of a one-step tris-*O*-MOM-protection in ribonucleoside synthesis. Longer reaction times result in the production of a tetrakis-MOM derivative, by TLC and NMR analysis.

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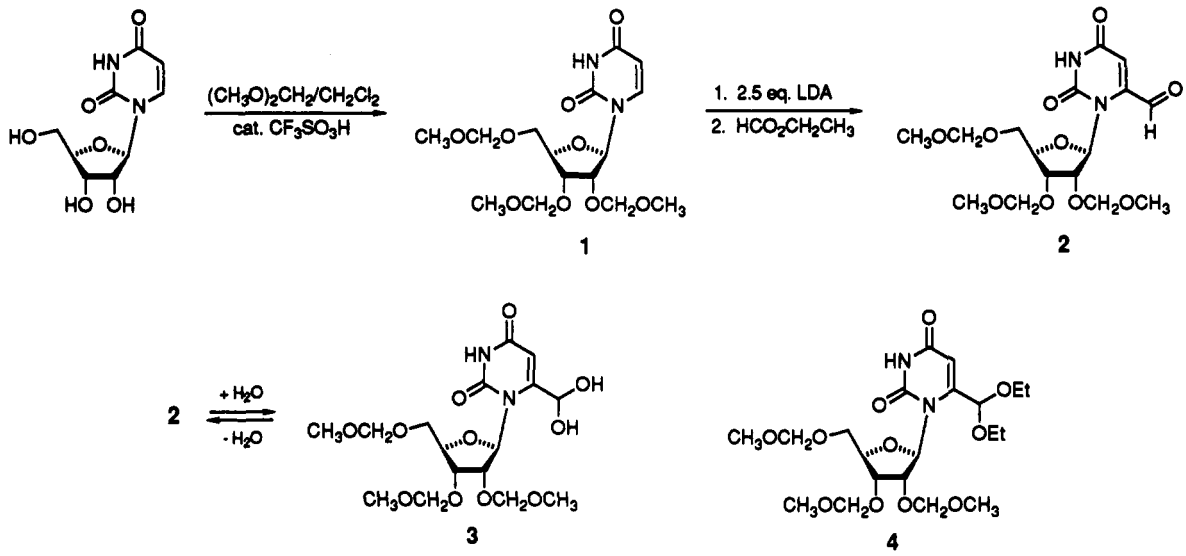
Scheme I



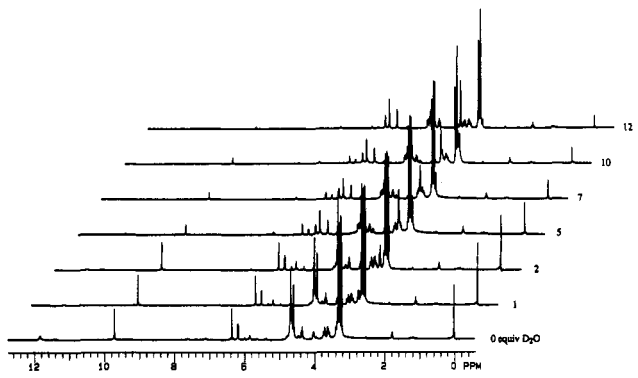


Figure 1. Effect of the incremental addition of 12 equiv of D_2O upon the ^1H NMR spectrum (300 MHz) of **2** in $(CD_3)_2SO$ solution.

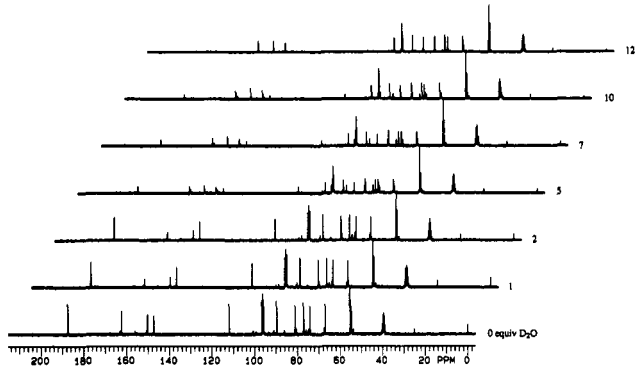
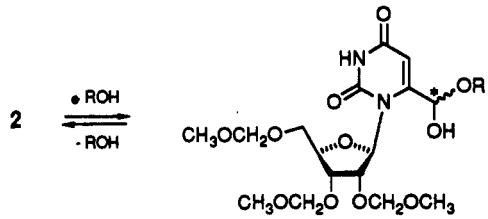


Figure 2. Effect of the incremental addition of 12 equiv of D_2O upon the ^{13}C NMR spectrum (75 MHz) of **2** in $(CD_3)_2SO$ solution.

nostic two-proton hydrate OH doublet at δ 7.23 associated by a 5.9-Hz coupling constant to a one-proton hydrate CH triplet at δ 5.64. Finally, the ^1H and ^{13}C NMR spectra of **2** and **3** labeled with 97% ^{13}C at the aldehyde and hydrate group, respectively, revealed single- and multiple-bond homonuclear and heteronuclear coupling constants in the expected ranges.

The formyl substituent of **2** is thus easily hydrated in much the same manner as that in other electron-deficient aldehydes such as Cl_3CCHO (chloral),[6] HO_2CCHO (glyoxylic acid),[7] or certain formylated nitrogen heterocycles.[8] Indeed, evidence for the strong electron-withdrawing ability of a 1-alkyluracil ring upon a C6 substituent can be found in the exceedingly low aqueous pK_{a1} value of 0.7 reported for 1-methyluracil-6-carboxylic acid (1-methylorotic acid).[9] A likely source of this electron-withdrawing ability is the C4 carbonyl moiety transmitting its effects via the C5,C6-double bond; in this way, compound **2** can be viewed as a vinylogous α-dicarbonyl compound.[10]

Importantly, the formation of hydrate **3** was shown to be reversible. Thus, **2** was completely regenerated upon treatment of a $(CD_3)_2SO$ solution of **3** with 4-Å molecular sieves, by ^1H NMR. In addition, treatment of **2** in $(CD_3)_2SO$ solution with absolute MeOH or EtOH each afforded a diastereomeric mixture of hemiacetal nucleosides (**5a,b** and **6a,b**, respectively) produced in a 1:1 ratio due to the lack of stereofacial selectivity in addition of these alcohols to the aldehyde moiety of **2**. Adducts **5** and **6** were characterized solely on the basis of their NMR spectral features.



5a,b R = Me
6a,b R = Et

Removal of the MOM-protecting groups of **2** was effected in aqueous CF_3CO_2H at room temperature, and the single deprotection product was isolated as a dry solid by lyophilization. Based upon the reactivity elucidated for **2**, we realized that the uridine-6-carboxaldehyde thus obtained might exist in several possible structural forms, any one or a combination of which might exist in the solid

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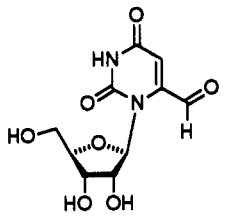
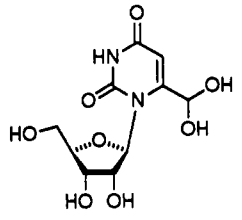
(9) Fox, J. J.; Yung, N.; Wempen, I. *Biochim. Biophys. Acta* **1957**, *23*, 295.

(10) We thank Prof. Gordon W. Gribble of Dartmouth College for assistance with this analysis.

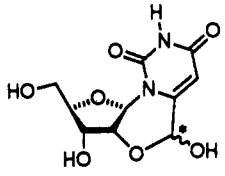
state, or in aqueous or nonaqueous solution. The first two forms considered were the free aldehyde structure A and the *gem*-diol structure B, and although the former was thought unlikely to be the *major* structure in any state, the latter was predicted to be a major component in aqueous solution. In addition to these, diastereomers of the 2′- or 5′-cyclic hemiacetal structures C or D were



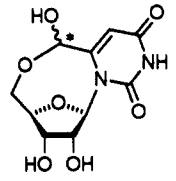
A



B



C



D

considered, but 3′-cyclic hemiacetal structures were eliminated on the basis of severe ring strain. We found uridine-6-carboxaldehyde to exist as a 1:10 mixture of structures in $(CD_3)_2SO$ solution but as a 1:2 mixture of structures in D_2O solution. A detailed NMR spectral analysis of each of these solutions was performed, and all four structures were assigned. The minor component (9%) in $(CD_3)_2SO$ solution was easily identified as the free aldehyde structure A according to the presence of a singlet aldehydic ^1H resonance at δ 9.85. As expected, the major component (67%) in D_2O solution was identified as the *gem*-diol structure B, by virtue of the fact that the chemical shift values of its aglycon ^1H and ^{13}C signals were virtually identical to those recorded for a D_2O solution of tri-*O*-protected hydrate 3. Surprisingly, we found *both* the major component (91%) in $(CD_3)_2SO$ solution *and* the minor component (33%) in D_2O solution each to be a diastereomer of the 5′-cyclic hemiacetal structure D. A definitive assignment of the 5′-cyclic structures in both solutions was made possible by the observation of a strong three-bond long-range heteronuclear correlation between the hemiacetal methine carbon atom and the 5′-methylene hydrogen atoms. The absolute configuration of the chiral hemiacetal methine carbon in these two structures has not yet been determined, nor has it been determined whether or not the same diastereomer of D is present in $(CD_3)_2SO$ and D_2O solution. We believe that formation of a 5′-cyclic structure D occurs preferentially to that of a 2′-cyclic C due to the "anomeric" effect,[11] in which an axially oriented electronegative substituent α to a ring oxygen atom is dipole-stabilized with respect to its equatorially oriented counterpart.

Conclusion

Our study reveals the strong electron-withdrawing effect that a 1-alkylpyrimidine-2,4-dione ring exerts upon a C6 substituent, which in the case of the protected 6-formyluridine derivative 2 renders the aldehyde moiety susceptible to the 1,2-addition of weakly nucleophilic species. We suggest that the instability associated with the 6-formyluridines reported to date is a direct result of such a 1,2-addition of, for example, propitious water in an NMR sample, a reaction likely to be catalyzed by the trace amounts of DCl known to develop readily in deuteriochloroform. Removal of the protecting groups from 2 has afforded a ribonucleoside compound with a rare physicochemical property similar to that of 5-azauridine, which exists predominantly as a cyclonucleoside formed by the reversible addition of the ribosyl 5′-hydroxyl group to the heterocyclic aglycon.[12] The unusual susceptibility of the formyl group to 1,2-additions may confer interesting biological and/or pharmacological properties upon deblocked uridine-6-carboxaldehyde and/or its sugar-modified derivatives. For example, the pyrimidine nucleotide-utilizing enzymes thymidylate synthetase (EC 2.1.1.6)[13] and ribonucleoside diphosphate reductase (EC 1.17.4.1)[14] are known to position a nucleophilic amino acid residue (a Cys-sulfhydryl group in each case) near the C6 position of the pyrimidine ring during catalysis. We are now working to obtain uridine-6-carboxaldehyde in a form suitable for X-ray crystallographic analysis and also to prepare the 2′-deoxyribofuranosyl and arabinofuranosyl derivatives of this compound for chemical and biological studies.

Experimental Section

Materials and Methods. Melting points were determined on a capillary melting point apparatus and are uncorrected. Radial preparative-layer chromatography was performed on a Chromatotron instrument (Harrison Research, Inc., Palo Alto, CA) using Merck silica gel 60 with fluorescent indicator as the adsorbent. Lyophilizations were done using a benchtop freeze-dryer apparatus. Tetrahydrofuran was dried by distillation from sodium under argon using benzophenone ketyl as indicator. Ethyl formate, triethyl orthoformate, dichloromethane, and dimethoxymethane were distilled from P_2O_5 under argon. Diisopropylamine was distilled from CaH_2 under dry argon. ^1H NMR spectra were recorded at 300 or 500 MHz, and ^{13}C NMR spectra were recorded at 75 MHz. These spectra were recorded with TMS or sodium (trimethylsilyl)propanesulfonate (both δ = 0.0 for ^1H) and $CDCl_3$ (δ = 77.0 for ^{13}C) or $(CD_3)_2SO$ (δ = 39.5 for ^{13}C) as internal reference. Short- (140-Hz-optimized) and long-range (7.5- or 10-Hz-optimized) 2D $^1H/^{13}C$ heteronuclear correlation (HETCOR) and COSY spectra were obtained on our 300-MHz instrument. Optimal long-range HETCOR results were obtained when the probe was tuned to each specific NMR sample; 97% ethyl formate-^{13}C was obtained from Isotec, Inc. Butyllithium in hexanes, ethyl formate, dimethoxymethane, and diisopropylamine were purchased from the Aldrich Chemical Co. The butyllithium was titrated with diphenylacetic acid in dry THF solution at 0 °C. Elemental microanalyses were performed by Tom McCarthy at the University of Illinois. Mass spectral analyses were obtained from Richard Milberg and his staff on the Mass Spectrometry facility at the University of Illinois.

2′,3′,5′-Tris-*O*-(methoxymethyl)uridine (1). A suspension of 2.5 g (10.2 mmol) of uridine in 1 L of 50% $CH_2(OCH_3)_2/CH_2Cl_2$ under argon was treated with 4 drops (ca. 0.2 mL) of CF_3SO_3H via Pasteur pipette. The reaction mixture was stirred for 4 h at room temperature, and the solution was quenched with ca. 0.4 mL of concd NH_4OH, dried (Na_2SO_4), and rotary evaporated to an oil, which was purified by column chromatography (4% CH_3OH/CH_2Cl_2 as eluent) to afford 2.47 g (65%) of 1 as an oil

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(13) Friedkin, M. *Advances in Enzymology*; Meister, A., Ed.; Wiley Interscience: New York, 1973; Part 38, p 235.

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which solidified upon rigorous pumping: mp 86–90 °C (Et_2O/pentane); ^1H NMR ($(CD_3)_2SO$) δ 11.4 (bs, exchanges with D_2O, 1 H, NH), 7.78 (d, J = 8.1 Hz, 1 H, H6), 5.90 (d, J = 5.1 Hz, 1 H, H1′), 5.71 (d, J = 8.1 Hz, 1 H, H5), 4.7–4.6 (m, 6 H, three CH_2OCH_3), 4.33 (m, 1 H, H2′), 4.18–4.17 (m, 2 H, H3′ and H4′), 3.70 (dd, $J_{5'a-5'b}$ = 33.5 Hz, $J_{4'-5'}$ = 9.9 Hz, 2 H, 5′-CH_2), 3.30 (s, 6 H, two CH_2OCH_3), 3.24 (s, 3 H, CH_2OCH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 163.1 (C4), 150.6 (C2), 140.0 (C6), 102.0 (C5), 96.0, 95.5 (three CH_2OCH_3), 86.8 (C1′), 81.3 (C4′), 76.7 (C2′), 73.7 (C3′), 66.4 (C5′), 55.1 and 54.9 (three CH_2OCH_3); long-range correlations observed in a 10-Hz-optimized ^1H–^{13}C HETCOR experiment were *C2/H6*, *C4/H6*, *C5/H6*, *C6/H5*, *C2′*/CH_2OCH_3, *C3′*/CH_2OCH_3, *C5′*/CH_2OCH_3, CH_2OCH_3/CH_2OCH_3, *C4′*/5′-CH_2, and *C3′*/5′-CH_2; low-resolution ACE (alternating CI/EI) mass spectrum, CI(CH_4) m/z 377.3 (MH^+). Anal. Calcd for $C_{15}H_{24}N_2O_9$: C, 47.87; H, 6.43; N, 7.44. Found: C, 48.02; H, 6.44; N, 7.42.

2′,3′,5′-Tris-*O*-(methoxymethyl)uridine-6-carboxaldehyde (2). A solution of 1.07 g (2.8 mmol) of 1 in 10 mL of anhydrous THF was added dropwise to a solution of freshly prepared LDA (8.5 mmol) in 10 mL of anhydrous THF at −78 °C under argon. After 3 h at −78 °C, HCO_2Et (0.56 mL, excess) was added all at once, and the reaction mixture was allowed to warm to room temperature. The reaction mixture was quenched by the addition of HOAc (0.5 mL, 8.5 mmol). Analysis by TLC (2,4-DNP visualization) gave evidence of desired product along with starting material. The reaction mixture was filtered, and the filtrate was rotary evaporated to give a mixture which was purified by radial chromatography (4% CH_3OH/CH_2CL_2 as eluent) to afford 569 mg (50%, 73% based upon recovered starting material) of 2 as an oil: ^1H NMR ($(CD_3)_2SO$) δ 11.83 (1 H, bs, exchanges with D_2O, NH), 9.68 (s, 1 H, CHO), 6.34 (s, 1 H, H5), 6.16 (d, J = 3.6 Hz, 1 H, H1′), 4.67–4.62 (m, 5 H, H2′ and two CH_2OCH_3), 4.60 (s, 2 H, CH_2OCH_3), 4.32 (m, 1 H, H3′), 4.40 (m, 1 H, H4′), 3.70–3.60 (m, 2 H, 5′-CH_2), 3.32 (s, 3 H, CH_2OCH_3), 3.30 (s, 3 H, CH_2OCH_3), 3.25 (s, 3 H, CH_2OCH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 187.4 (CHO), 162.3 (C4), 150.2 (C2), 147.2 (C6), 111.9 (C5), 96.5, 96.0, and 96.6 (three CH_2OCH_3), 89.5 (C1′), 80.9 (C4′), 77.0 (C2′), 74.0 (C3′), 67.0 (C5′), 55.2, 55.1, and 54.7 (three CH_2OCH_3); long-range correlations observed in a 10-Hz-optimized ^1H–^{13}C HETCOR experiment were *C6/H5*, *CHO/H5*, *C2/H1′*, *C2′*/CH_2OCH_3, *C3′*/CH_2OCH_3, *C5′*/CH_2OCH_3, CH_2OCH_3/CH_2OCH_3; ^1H NMR (D_2O) δ 6.13 (s, 1 H, H5), 6.05 (d, J = 3.0 Hz, 1 H, H1′), 5.95 (s, 1 H, $CH(OD)_2$), 4.98–4.95 (m, 1 H, H2′), 4.83–4.74 (m, 4 H, two CH_2OCH_3), 4.71 (s, 2 H, CH_2OCH_3), 4.59–4.54 (m, 1 H, H3′), 4.18–4.12 (m, 1 H, H4′), 3.93–3.72 (m, 2 H, 5′-CH_2), 3.45 (s, 3 H, CH_2OCH_3), 3.39 (s, 6 H, two CH_2OCH_3); ^{13}C NMR (D_2O) δ 168.3 (C4), 158.5 and 154.2 (C2/C6), 103.1 (C5), 99.3, 99.0, and 98.9 (three CH_2OCH_3), 93.9 (C1′), 88.7 ($CH(OD)_2$), 83.2 (C4′), 79.4 (C2′), 77.5 (C3′), 69.7 (C5′), 58.5, 58.4, and 57.8 (three CH_2OCH_3); low-resolution FD mass spectrum, m/z 404 (M^+), 405 (MH^+); UV λ_{max} nm (ε × 10^3) (dioxane) 294 (6.5), 259 (4.3), 237 (4.5), 233 (4.1), 222 (4.0), 211 (2.2); (EtOH) 261 (10.6), 255 (11.1), 249 (8.8), 209 (38.4); (pH 1) 260 (10.2), 254 (10.4), 248 (8.4), 208 (44.1); (pH 7) 260 (11.1), 254 (11.3), 249 (9.2), 208 (44.2); (pH 11) 260 (7.0), 254 (7.3), 248 (6.7), 243 (6.1), 224 (6.2); IR (Nujol, NaCl) ν 1731 cm^{-1} (aldehydic C=O stretch). Anal. Calcd for $C_{16}H_{24}N_2O_{10}$: C, 47.52; H, 5.98; N, 6.93. Found: C, 47.58; H, 6.06; N, 6.57.

2′,3′,5′-Tris-*O*-(methoxymethyl)uridine-6-carboxaldehyde-*formyl*-^{13}C. This was prepared by substituting 97% ethyl formate-^{13}C for the HCO_2Et in the above procedure. Selected ^1H and ^{13}C NMR data in $(CD_3)_2SO$ solution: $^1J_{CHO-CHO}$ = 198 Hz, $^3J_{CHO-H5}$ = 3.9 Hz, $^1J_{CHO-C6}$ = 52 Hz, $^2J_{CHO-C5}$ = 5.4 Hz, $^3J_{CHO-C4}$ = 6.2 Hz, $^3J_{CHO-C2}$ = 2.7 Hz.

Hydration of 2. A solution of 82.1 mg (0.2 mmol) of 2 in 0.5 mL of $(CD_3)_2SO$ was prepared. Analysis of the ^1H and ^{13}C NMR spectra of this solution recorded after the addition of 1, 2, 5, 7, 10 and 12 equiv of D_2O or H_2O indicated the progressive formation of a single new product. The spectral data were consistent with the formation of **6-(dihydroxymethyl)-2′,3′,5′-tris-*O*-(methoxymethyl)uridine** (3, hydrated-2). Data obtained from addition of D_2O to 2: ^1H NMR ($(CD_3)_2SO$) δ 6.01 (d, 1 H, J = 2.7 Hz, H1′), 5.94 (s, 1 H, H5), 5.74 (s, 1 H, $CH(OD)_2$), 4.79 (m, 1 H, H2′), 4.76–4.65 (m, 4 H, two CH_2OCH_3), 4.65 (s, 2 H, CH_2OCH_3), 4.47 (m, 1 H, H3′), 4.00 (m, 1 H, H4′), 3.81–3.59 (m, 2 H, 5′-CH_2), 3.36 (s, 3 H, CH_2OCH_3), 3.30 (s, 6 H, two CH_2OCH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 165.2 (C4), 157.2 (C6), 151.7 (C2), 100.6 (C5), 97.0 (three CH_2OCH_3), 92.1 (C1′), 87.0 ($CH(OH)_2$), 81.5 (C4′), 77.1 (C2′), 75.5 (C3′), 68.3 (C5′), 56.4 and 55.9 (three CH_2OCH_3); long-range correlations observed in a 10-Hz-optimized ^1H–^{13}C HETCOR experiment were *C2/H1′*, *C5/CH*$(OH)_2$, *C6/H1′*, *C2/H1′*, *C3′/H2′*, *C5′*/CH_2OCH_3, and CH_2OCH_3/CH_2OCH_3.

Selected ^1H NMR data obtained from addition of H_2O to 2: ^1H NMR ($(CD_3)_2SO$) δ 11.43 (bs, 1 H, NH), 7.23 (bd, J = 5.9 Hz, 2 H, $CH(OH)_2$), 5.99 (d, J = 2.1 Hz, 1 H, H1′), 5.85 (s, 1 H, H5), 5.64 (bt, J = 5.9 Hz, 1 H, $CH(OH)_2$).

Dehydration of a $(CD_3)_2SO$ solution of 3 (prepared by the addition of 12 equiv of D_2O to 2) with 4-Å molecular sieves gave a solution which revealed no ^1H NMR signals previously assigned to 3 but only those originally assigned to compound 2.

6-(Dihydroxymethyl)-2′,3′,5′-tris-*O*-(methoxymethyl)uridine-*hydrate*-^{13}C. This was prepared by hydrating 2-*formyl*-^{13}C according to the above procedure. Selected ^1H and ^{13}C NMR data in $(CD_3)_2SO$ solution: $^1J_{CH(OH)_2-CH(OH)_2}$ = 162 Hz, $^3J_{CH(OH)_2-H5}$ = 2.7 Hz, $^1J_{CH(OH)_2-C6}$ = 58 Hz, $^2J_{CH(OH)_2-C5}$ = 0.0 Hz, $^3J_{CH(OH)_2-C4}$ = 5.1 Hz, $^3J_{CH(OH)_2-C2}$ = 0.0 Hz.

2′,3′,5′-Tris-*O*-(methoxymethyl)uridine-6-carboxaldehyde Diethyl Acetal (4). A solution of 117 mg (0.29 mmol) of 2 in 5 mL of $(EtO)_3CH$ was treated with CF_3SO_3H (5 μL, 0.06 mmol). The reaction mixture was stirred for 30 min at room temperature and then was quenched with 0.2 mL of concd NH_4OH. The solution was dried ($MgSO_4$) and evaporated to dryness, and the residue was purified by radial chromatography (4% CH_3OH/CH_2Cl_2 as eluent) to give 129 mg (89%) of 4 as an oil: ^1H NMR ($(CD_3)_2SO$) δ 11.5 (bs, exchanges with D_2O, 1 H, NH), 5.84 (s, 1 H, H5), 5.83 (d, J = 3.3 Hz, 1 H, H1′), 5.33 (s, 1 H, acetal-CH), 4.79–4.77 (m, 1 H, H2′), 4.69–4.62 (m, 4 H, two CH_2OCH_3), 4.57 (s, 2 H, CH_2OCH_3), 4.40 (m, 1 H, H3′), 3.93 (m, 1 H, H4′), 3.72–3.52 (m, 6 H, 5′-CH_2 and two OCH_2CH_3), 3.33–3.25 (m, 9 H, three CH_2OCH_3), 1.17 (t, J = 7.0 Hz, 6 H, two OCH_2CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 162.4 (C4), 150.8 and 150.5 (C2/C6), 102.2 (C5), 98.4 (acetal-CH), 95.8–95.6 (three CH_2OCH_3), 91.0 (C1′), 80.7 (C4′), 75.6 (C2′), 74.5 (C3′), 67.4 (C5′), 63.0 and 62.5 (two OCH_2CH_3), 55.1 and 54.6 (three CH_2OCH_3), 14.7 (two OCH_2CH_3); long-range correlations observed in a 10-Hz-optimized ^1H–^{13}C HETCOR experiment were *C2′/H1′*, *C2′*/CH_2OCH_3, *C3′*/CH_2OCH_3, *C5′*/CH_2OCH_3, OCH_2CH_3/acetal-*CH*, CH_2OCH_3/CH_2OCH_3, and OCH_2CH_3/OCH_2CH_3; low-resolution FAB mass spectrum, m/z 479.2 (MH^+); high-resolution FAB mass spectrum 479.2232 ($C_{20}H_{35}N_2O_{11}$ requires 479.2241). Anal. Calcd for $C_{20}H_{34}N_2O_{11}$: C, 50.20; H, 7.16; N, 5.85. Found: C, 49.73; H, 7.05; N, 5.73.

2′,3′,5′-Tris-*O*-(methoxymethyl)uridine-6-carboxaldehyde Methyl and Ethyl Hemiacetals (5a,b and 6a,b). Solutions of 2 (50 mg, 0.12 mmol) in 0.5 mL of dry $(CD_3)_2SO$ were treated with absolute MeOH or absolute EtOH (56 μL or 90 μL, respectively, 1.5 mmol) and then were allowed to equilibrate for 5 h at room temperature to allow for the complete formation of hemiacetals **5a,b** and **6a,b**, respectively. Data for **5a,b**: ^1H NMR ($(CD_3)_2SO$) δ 5.88–5.78 (m, 2 H, H1′ and H5), 5.35 (s, 1 H, hemiacetal-CH), 4.73–4.54 (m, 7 H, H2′ and CH_2OCH_3), 4.50–4.45 (m, 1 H, H3′), 3.92 (m, 1 H, H4′), 3.74–3.40 (m, 2 H, 5′-CH_2), 3.33–3.25 (m, 9 H, three CH_2OCH_3), 3.18 (s, 3 H, hemiacetal-OCH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 163.2 (C4), 152.3 and 153.2 (each C2/C6), 150.9 (C2/C6), 100.9 (C5), 96.0, 95.8, and 95.7 (three CH_2OCH_3), 93.6 and 93.2 (each hemiacetal-CH), 91.4 and 91.3 (each C1′), 80.8 and 80.7 (each C4′), 76.3 and 75.9 (each C2′), 74.7 (C3′) 67.6 and 67.5 (each C5′), 55.2 and 54.7 (three CH_2OCH_3), 53.8 (hemiacetal-OCH_3). Data for **6a,b**: ^1H NMR ($(CD_3)_2SO$) δ 5.87–5.80 (m, 2 H, H1′ and H5), 5.43 and 5.42 (each bs, 1 H, each hemiacetal-CH), 4.67–4.54 (m, 7 H, H2′ and three CH_2OCH_3), 4.40 (m, 1 H, H3′), 3.95 (m, 1 H, H4′), 3.75–3.65 (m, 2 H, 5′-CH_2), 3.45 (q, J = 6.9 Hz, 2 H, OCH_2CH_3), 3.30 and 3.25 (m, 9 H, CH_2OCH_3), 1.16 (t, J = 6.9 Hz, 3 H, OCH_2CH_3); ^{13}C NMR ($(CD_3)_2SO$) δ 162.8 (C4), 153.7 and 153.6 (each C2/C6), 150.6 (C2/C6), 100.6 (C5), 95.8 (three CH_2OCH_3), 92.2 and 91.7 (each hemiacetal-CH), 91.2 and 90.8 (each C1′), 80.7 and 80.6 (each C4′), 75.9 and 75.5 (each C2′), 74.6 (C3′), 67.6 and 67.5 (each C5′), 62.1 and 62.0 (each OCH_2CH_3), 55.2 and 54.6 (three CH_2OCH_3), 14.8 and 14.7 (each OCH_2CH_3).

Uridine-6-carboxaldehyde (A–D). A solution of 170 mg (0.41 mmol) of 2 in 4.4 mL of 50% aqueous CF_3CO_2H was stirred at room temperature for 24 h. The reaction mixture was rotary evaporated, and traces of CF_3CO_2H were removed by repetitive azeotropic rotary evaporation with water (3 × 50 mL). The residue

was purified by radial chromatography (20% CH_3OH/CH_2Cl_2 as eluent), and the product was redissolved in 250 mL of water. Lyophilization for 48 h afforded 103 mg (91% of a nonhydrated structure) of product as an off-white powder: low-resolution FAB mass spectrum (using dithiothreitol in methanol), m/z 460.8 (100, $MH^+ + H_2 + CH_3OH$ + DTT), 306.8 (93, $MH^+ + H_2 + CH_3OH$), 272.8 (9, MH^+). Recrystallization from water afforded needles: mp 182-184 °C; low-resolution FD mass spectrum, m/z 272 (M^+), 254 ($M^+ - H_2O$); UV λ_{max} nm ($\epsilon \times 10^3$) (H_2O) 265 (12.4), 204 (12.1); (pH 1) 264 (12.3), 208 (11.0); (pH 7) 263 (11.5), 209 (8.6); (pH 11) 264 (10.5), 230 (9.2), 214 (7.4). Analysis of this material revealed that it exists as a 1:10 mixture of structures in $(CD_3)_2SO$ solution, but as a 1:2 mixture of structures in D_2O solution.

A (9% component in $(CD_3)_2SO$): ^1H NMR ($(CD_3)_2SO$) δ 9.85 (s, 1 H, CHO).

D (91% component in $(CD_3)_2SO$): ^1H NMR ($(CD_3)_2SO$) δ 11.38 (s, exchanges with D_2O, 1 H, NH), 7.22 (d, J = 6.3 Hz, exchanges with D_2O, 1 H, hemiacetal-OH), 6.28 (d, J = 4.5 Hz, 1 H, H1'), 5.91 (s, 1 H, H5), 5.80 (d, J = 6.3 Hz, collapses to a singlet upon addition of D_2O, 1 H, hemiacetal-CH), 5.29 (d, J = 6.6 Hz, exchanges with D_2O, 1 H, 2'-OH), 5.06 (d, J = 4.2 Hz, exchanges with D_2O, 1 H, 3'-OH), 4.31 (m, 1 H, H2'), 4.21 (bs, 1 H, H4'), 3.97 (m, 1 H, H3'), 3.85 (s, 2 H, 5'-CH_2); ^{13}C NMR ($(CD_3)_2SO$) δ 162.6 (C4), 153.7 and 151.6 (C2/C6), 100.9 (C5), 94.5 (hemiacetal-CH), 93.1 (C1'), 86.1 (C4'), 77.7 (C2'), 73.5 (C3'), 69.3 (C5').

Long-range correlations observed in a 7.5-Hz-optimized ^1H-^{13}C HETCOR experiment were hemiacetal-*C*H/5'-*C*H_2, *C*1'/*H*4', *C*2'/*H*4', *C*3'/*H*2', *C*3'/5'-*C*H_2 for D.

B (67% component in D_2O): ^1H NMR (D_2O) δ 6.12 (s, 1 H, H5), 5.97 (s, 1 H, $CH(OD)_2$), 5.94 (d, J = 2.7 Hz, 1 H, H1'), 4.75 (m, 1 H, H2'), 4.45 (m, 1 H, H3'), 3.95 (m, 1 H, H4'), 3.87-3.72 (m, 2 H, 5'-CH_2); ^{13}C NMR (D_2O) δ 168.4 (C4), 158.7 and 154.2 (C2/C6), 103.0 (C5), 95.0 (C1'), 88.6 (*C*H(OD)$_2$), 86.1 (C4'), 74.6 (C2'), 71.9 (C3'), 64.2 (C5').

D (33% component in D_2O): ^1H NMR (D_2O) δ 6.41 (d, J = 4.5 Hz, 1 H, H1'), 6.23 (s, 1 H, H5), 6.10 (s, 1 H, hemiacetal-CH), 4.62 (m, 1 H, H2'), 4.48 (m, 1 H, H4'), 4.30 (m, 1 H, H3'), 4.14-3.99 (m, 2 H, 5'-CH_2); ^{13}C NMR (D_2O) δ 167.9 (C4), 157.1 and 155.1 (C2/C6), 103.8 (C5), 97.2 (hemiacetal-CH), 96.2 (C1'), 89.1 (C4'), 80.4 (C2'), 76.5 (C3'), 57.0 (C5').

Long-range correlations observed in a 10-Hz-optimized ^1H-^{13}C HETCOR experiment were *C*2'/*H*1', *C*4'/5'-*C*H_2, and *C*3'/*H*4' for B, and hemiacetal-*C*H/5'-*C*H_2 and *C*3'/5'-*C*H_2 for D.

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Supplementary Material Available: ^1H and ^{13}C NMR spectral data for 1 and 2 in $CDCl_3$ and for 4 in $CDCl_3$; long-range HETCOR NMR spectral data for 1 and 2 in $CDCl_3$; and COSY and short- and long-range HETCOR NMR spectral plots of 1, 2, 3, and 4 in $(CD_3)_2SO$ solution and for uridine-6-carboxaldehyde in both D_2O and $(CD_3)_2SO$ solution (20 pages). Ordering information is given on any current masthead page.

Ene Reactions of Dialkyl Dioxosuccinate Esters

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The ene reactions of dimethyl dioxosuccinate (1) and of diethyl dioxosuccinate (2) with olefins give the expected addition products. The reactions of 1 with vinyl ethers or an eneamine provide the cyclopentenones 15 and 16 in a sequence which is consistent with an ene reaction followed by a cyclization. The tin tetrachloride catalyzed conversion of 6 to 17 provides an example of a formal type II ene reaction to give a cyclopentyl ring. However, the stereochemistry of 17 suggests the reaction involves a stepwise ionic process.

The ene reaction, once considered a novel process, has been developed into a reaction of considerable synthetic value with catalyzed and intramolecular carbon-carbon bond formations receiving serious attention in recent years.[1] Our interest in the mechanism of the ene reaction led us to study dimethyl dioxosuccinate, a previously uninvestigated eneophile.[2] In this paper, we report that dialkyl dioxosuccinate esters undergo the ene reaction with a variety of olefins, and that five-membered carbocycles can be obtained from the initial products in certain cases.[3,4]

Preparation of Dialkyl Dioxosuccinate Esters. The syntheses of dimethyl and diethyl dioxosuccinate (1 and 2, respectively) were achieved by the acid-catalyzed dehydration and esterification of readily available dihydroxy tartaric acid disodium salt with the appropriate alcohol following the procedures of Fox and of Boger.[5] Although these orange-yellow diketo diesters form viscous oils or

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(4) Efforts to make cyclopentenes by type II ene reactions usually have led to products in which the ring is formed but nucleophilic substitutions or rearrangements occur. This can be taken to suggest the reactions are not ene processes.[3a,c-e] Precedent for the conversion of 6 to 17 and 18 is provided by the work of Andersen et al.[3d]

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