Facile Unmasking of Ethenylated Isocytosines *via* Diacetoxylation with Lead Tetraacetate

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Treatment of ethenylated isocytosines, imidazolo[1,2-*a*]pyrimidine-5(1*H*)-one (2) and -7(8*H*)-one (3), with lead tetraacetate (LTA) in glacial acetic acid followed by alkaline hydrolysis resulted in the smooth removal of the ethenyl group to give isocytosine (1) in high yields. The unmasking of 2 by LTA to 1 was compared with the results using iodosylbenzene diacetate and *N*-bromosuccinimide.

Keywords imidazolo[1,2-*a*]pyrimidine-5(1*H*)-one; imidazolo[1,2-*a*]pyrimidine-7(8*H*)-one; lead tetraacetate; alkaline hydrolysis; unmasking; isocytosine

Ethenylation of the amidine or guanidine moiety in adenosines, cytidines, and guanosines has been extensively investigated from the biological points of view, *i.e.*, fluorescent modification of nucleic acids[1] and mutagenicity of vinyl halides, halogenoethylene oxides, or halogenoacetaldehydes.[2] However, there have been only a few reports concerning removal of the ethenyl group. For example, the unmasking of ethenylated adenosine 3′,5′-cyclic phosphates was achieved by bromination with *N*-bromosuccinimide (NBS) followed by alkaline hydrolysis,[3] although its versatility and conversion yields are unsatisfactory.

We describe a smooth unmasking of ethenylated isocytosines, imidazolo[1,2-*a*]pyrimidine-5(1*H*)-one (**2**) and -7(8*H*)-one (**3**), to isocytosine (**1**) which involves diacetoxylation with lead tetraacetate (LTA) and subsequent alkaline hydrolysis. The reactions for removing the ethenyl group in **2** and **3** proceed under mild conditions without accompanying undesirable reactions. The present method can be applied in principle to the unmasking of the ethenyl protection for the guanidine moiety in guanines, biopterin, and folic acid.

Warming a mixture of **1** and bromoacetaldehyde diethylacetal in H_2O at 70 °C for 2 d gave two separable isomers of ethenylated isocytosines, **2** and **3**, in 54 and 37% yields, respectively. Similar results (yields and product ratio) were obtained upon employment of chloroacetaldehyde in place of bromoacetaldehyde diethylacetal. The structures of **2** and **3** were confirmed by comparison with spectral data previously reported[4,5] and by nuclear Overhauser effect (NOE) experiments (see Experimental section).

A solution of **2** and a slight excess of LTA in glacial AcOH was stirred at room temperature overnight. After removal of the solvent, the resulting residue was treated with dilute $NaHCO_3$ at room temperature for 2 h. The quantitative formation of **1** in this reaction was proved by thin layer chromatography (TLC) analysis and proton nuclear magnetic resonance (^{1}H-NMR) spectral measurement of the reaction mixture.

Mechanistic insights into the unmasking of **2** to **1** under the conditions employed were obtained as follows.

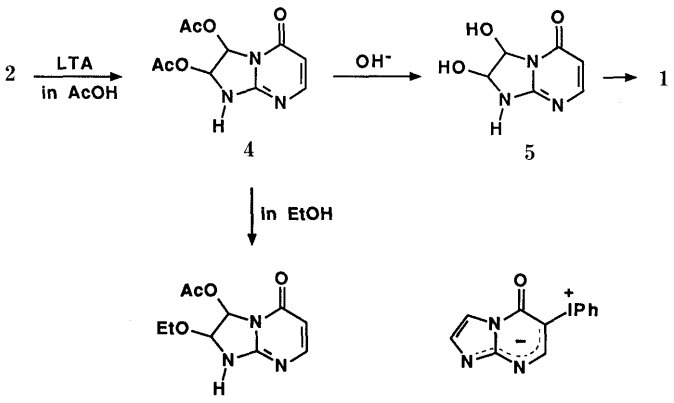
The ^{1}H-NMR spectral change during the reaction of **2** with LTA in CD_3CO_2D showed the smooth and quantitative conversion of **2** into 2,3-diacetoxy-2,3-dihydroimidazolo[1,2-*a*]pyrimidine-5(1*H*)-one (**4**) [δ 6.07 (1H, d, J=6.7 Hz, C_6-H), 6.11 (1H, s, C_2-H), 6.99 (1H, s, C_3-H), 7.70 (1H, d, J=6.7 Hz, C_7-H) ppm], which was not isolated in a pure state.[6] When the reaction mixture containing **4** in AcOH was diluted with EtOH and then stirred at room temperature for 1 h, 3-acetoxy-2-ethoxy-2,3-dihydroimidazolo[1,2-*a*]pyrimidine-5(1*H*)-one (**6**) was obtained as a crystalline product in a high yield. The structural proof of **6** rests upon its microanalytical results and spectral data (see the experimental section). These facts indicate that the attack of LTA occurs regioselectively on the imidazole ring of **2** and the product **4** is labile in a protic solvent, undergoing nucleophilic substitution at the C_2-position.

The alkaline hydrolysis of **4** resulted in the intermediary formation of 2,3-dihydroxy-2,3-dihydroimidazolo[1,2-*a*]-pyrimidine-5(1*H*)-one (**5**), as indicated by NMR spectroscopy: the ^{1}H-NMR spectrum of the mixture after the alkaline treatment of **4** for a short period showed the presence of two singlet signals [δ 5.19 (1H) and 5.72 (1H) in D_2O] assignable to the C_2- and C_3-protons of **5**, together with two doublet signals due to the C_6- and C_7-protons.

Taking the above facts and the well-demonstrated reactivities of LTA[7] into consideration, the reaction sequence for the conversion of **2** into **1** can be outlined as shown in Chart 2, which involves the intermediary

1 2 3

Chart 1



6 7

Chart 2

formation of **4** and **5**. Under analogous conditions, the isomeric ethenylated isocytosine (**3**) gave **1** quantitatively.

The unmasking of **2** leading to **1** was also observed in the reactions using iodosylbenzene diacetate (IBDA)[8] or NBS[3,9] in place of LTA. Employment of these reagents, however, did not give satisfactory results because of concurrent undesirable side-reactions: treatment of **2** with IBDA caused phenyliodosylation at its C_6-position to give a mesoionic compound (**7**) as a major product[10] and the reaction of **2** with NBS resulted in the formation of 6-bromoimidazolo[1,2-*a*]pyrimidine-5(1*H*)-one.

The present results provided a basis for the utilization of the ethenyl group as a protecting group of amidine and guanidine moieties in biologically important substances. Further explorations of this methodology are in progress.

Experimental

All melting points were determined on a Yanagimoto micro hot-stage apparatus and are uncorrected. Elemental analyses were performed by the microanalytical laboratory of our university. Spectroscopic measurements for the structural assignment of the products were performed with the following instruments: Infrared (IR) spectra with a Hitachi 215 spectrometer; Ultraviolet (UV) absorption spectra with a Shimadzu 260 spectrophotometer; ^{1}H-NMR spectra with a JEOL JNM-GX270 (270 MHz) Fourier translation (FT)-NMR spectrometer using tetramethylsilane as an internal standard; mass spectra (MS) and high-resolution mass spectra (HR-MS) with a JEOL JMS-D 300 machine operating at 70 eV. Thin-layer chromatographic (TLC) analyses were carried out on precoated Silicagel 60 F_{254} plates (Merck, Art 5715). Column chromatography was accomplished by using silica gel (Wakogel C-300).

Imidazolo[1,2-*a*]pyrimidine-5(1*H*)-one (2) and -7(8*H*)-one (3) A mixture of isocytosine (**1**) (HCl salt, 224 mg, 1.52 mmol) and bromoacetaldehyde diethylacetal (796 mg, 4.04 mmol) in H_2O (5 ml) was heated at 70 °C for 2 d. After neutralization with $NaHCO_3$ followed by removal of the solvent under reduced pressure, the resulting residue was subjected to column chromatography ($CHCl_3$: MeOH : AcOH = 200 : 20 : 1) to separate **2** (mp 222—3 °C, lit.[4] mp 222 °C) (110 mg, 54%) and **3** [mp 222 °C (dec.), lit.[5] mp 218—20 °C] (76 mg, 37%). The structures of **2** and **3** were confirmed by comparison of the spectral data (^{1}H-NMR, UV, and IR) with data previously reported and by NOE experiments [a strong NOE (in dimethylsulfoxide-d_6 (DMSO-d_6)) was observed between the C_3- (δ 7.43, d, J = 1.7 Hz) and C_5- (δ 8.38, d, J = 7.7 Hz) protons in **3** and no NOE was observed between the ethenyl protons and the C_6- or C_7-protons in **2**].

Similar results (yields of **2** and **3**; product ratio of **2** and **3** = 7 : 5) were obtained upon employment of chloroacetaldehyde (40% aqueous solution) in place of the bromoacetaldehyde.

Unmasking of 2 and 3 with LTA A solution of **2** (68 mg, 0.5 mmol) in glacial AcOH (5 ml) was treated with LTA (90% purity, 300 mg, 0.6 mmol). After stirring of the mixture at room temperature until the disappearance of **2** was complete (monitored by TLC, overnight), the resulting mixture was concentrated to 1/10 volume under reduced pressure and then poured into dilute $NaHCO_3$ solution (5 ml). The alkaline solution was stirred at room temperature for 2 h. TLC and ^{1}H-NMR spectral analyses of the reaction mixture after removal of the solvent showed the formation of a sole product. The product isolated by column chromatography ($CHCl_3$: MeOH = 5 : 1) (47 mg, 84%) was identical with authentic **1**.

A similar result (isolated yield of **1** = 87%) was obtained in the treatment of **3** (0.5 mmol) with LTA followed by alkaline hydrolysis under the conditions analogous to those used in the case of **2**.

2,3-Diacetoxy-2,3-dihydroimidazolo[1,2-*a*]pyrimidine-5(1*H*)-one (4) A mixture of **2** (2.7 mg, 0.02 mmol) and LTA (90% purity, 9.8 mg, 0.02 mmol) in CD_3CO_2D (0.5 ml) was allowed to stand at room temperature in a sealed NMR tube and the reaction was followed by NMR spectroscopy. The ^{1}H-NMR spectral change during the reaction showed a smooth conversion of two pairs of doublet signals [δ 6.20 (1H, J = 6.8 Hz), 8.04 (1H, J = 6.8 Hz), 7.44 (1H, J = 2.0 Hz), 7.79 (1H, J = 2.0 Hz) which are assignable respectively to the C_6-, C_7-, C_3-, and C_2-protons of **2**] into two singlet signals [δ 6.11 (1H), 6.99 (1H)] and a pair of doublet signals [δ 6.07 (1H, J = 6.7 Hz), 7.70 (1H, J = 6.7 Hz)] which are assignable to the four ring-protons of **4**, though two singlet signals due to the acetoxy groups of **4** were masked by the peaks of the solvent and LTA.

2,3-Dihydroxy-2,3-dihydroimidazolo[1,2-*a*]pyrimidine-5(1*H*)-one (5) After treatment of **2** (0.1 mmol) with LTA (0.1 mmol) in AcOH under the conditions described above followed by removal of the solvent, the residue was dissolved in dilute $NaHCO_3$ solution (5.0 ml). The alkaline solution was stirred at room temperature for 1 h and then evaporated to dryness. The ^{1}H-NMR spectrum (in D_2O) of the resulting mixture showed the presence of two singlet signals [δ 5.19 (1H), 5.72 (1H)] and a pair of doublet signals [δ 5.87 (1H, J = 6 Hz), 7.67 (1H, J = 6 Hz)] which are respectively assignable to the C_2-, C_3-, C_6-, and C_7-protons of **5**, together with a pair of doublet signals [δ 5.80 (1H, J = 6.8 Hz, C_5-H), 7.53 (1H, J = 6.8 Hz, C_6-H)] due to **1** (product ratio of **5**/**1** = 0.5).

3-Acetoxy-2-ethoxy-2,3-dihydroimidazolo[1,2-*a*]pyrimidine-5(1*H*)-one (6) A solution of **2** (13.6 mg, 0.1 mmol) in AcOH (1.0 ml) containing LTA (90% purity, 60 mg, 0.1 mmol) was stirred at room temperature overnight. The resulting mixture was diluted with EtOH (5 ml) and then the solution was stirred at room temperature for 1 h. TLC analysis of the reaction mixture showed the formation of a less polar compound (Rf = 0.6, $CHCl_3$: MeOH = 10 : 1) compared with the starting **2**, as a major product. Column chromatographic separation ($CHCl_3$: acetone = 10 : 1) of the mixture allowed isolation of **6** (17 mg, 71%). mp 135—136 °C (from Et_2O). *Anal.* Calcd for $C_{10}H_{13}N_3O_4 \cdot H_2O$: C, 46.69; H, 5.88; N, 16.34. Found: C, 47.08; H, 5.68; N, 16.46. MS *m/z*: 239 (M^+, 17%), 210 ($M^+ - Et$, 1%), 196 ($M^+ - Ac$, 8%), 193 ($M^+ - EtOH$, 8%), 179 (19%), 151 (100%). IR ν_{max}^{KBr}: 1760 (C=O), 1700 (C=O) cm^{-1}. UV λ_{max}^{MeOH}: 289 (ε 1.9 × 10^4), 218 (1.9 × 10^4) nm. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, $OCH_2C\underline{H}_3$), 2.14 (3H, s, OAc), 3.65—3.76 (2H, m, $OC\underline{H}_2CH_3$), 4.94 (1H, s, C_2-H), 5.88 (1H, d, J = 6.8 Hz, C_6-H), 6.75 (1H, s, C_3-H), 7.53 (1H, d, J = 6.8 Hz, C_7-H).

Reaction of 2 with IBDA A solution of **2** (68 mg, 0.5 mmol) and IBDA (197 mg, 0.6 mmol) in AcOH (2 ml) was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in dilute $NaHCO_3$ (5 ml) and then the solution was stirred at room temperature for 2 h. TLC analysis of the reaction mixture showed the formation of a polar compound (Rf = 0.42, $CHCl_3$: MeOH : AcOH = 16 : 6 : 3) as a major product, together with **1**. The mixture was separated by column chromatography ($CHCl_3$: MeOH = 5 : 1) to isolate **1** (identical with an authentic sample) (5 mg, 9%) and 6-phenyliodosylimidazolo-[1,2-*a*]pyrimidine-5(1*H*)-one (**7**) (105 mg, 62%). mp 211—213 °C (from MeOH). *Anal.* Calcd for $C_{12}H_8IN_3O$: C, 42.75; H, 2.39; N, 12.46. Found: C, 42.76; H, 2.48; N, 12.43. MS *m/z*: 337 (M^+, 18%), 261 (18%), 204 (IPh, 78%), 135 (12%), 77 (100%). IR ν_{max}^{KBr}: 1640 cm^{-1}. UV λ_{max}^{MeOH}: 304 (ε 1.1 × 10^4), 214 (3.0 × 10^4) nm. ^{1}H-NMR (DMSO-d_6) δ: 7.25 (1H, d, J = 1.5 Hz, C_3-H), 7.50—7.67 (3H, m, Ph-H), 7.57 (1H, d, J = 1.5 Hz, C_2-H), 8.05 (2H, br d, J = 7.3 Hz, Ph-H), 8.57 (1H, s, C_7-H).

Reaction of 2 with NBS A mixture of **2** (13.6 mg, 0.10 mmol) and NBS (19.7 mg, 0.11 mmol) in H_2O (3 ml) was stirred at room temperature for 1 h and then warmed at 80 °C for 1 h. After removal of the solvent, the residue was subjected to column chromatography ($CHCl_3$: MeOH = 50 : 1) to give **1** (5 mg, 49%), **2** (3 mg, 22%), and 6-bromoimidazolo[1,2-*a*]-pyrimidine-5(1*H*)-one (2 mg, 10%). HR-MS Calcd for $C_6H_4{}^{79}BrN_3O$: 212.9524. Found: 212.9510. MS *m/z*: 215 (96%), 213 (M^+, 100%), 187 (18%), 185 (18%), 134 ($M^+ - Br$, 10%), 106 (34%). ^{1}H-NMR (D_2O) δ: 7.45 (1H, d, J = 2 Hz, C_3-H), 7.62 (1H, d, J = 2 Hz, C_2-H), 8.33 (1H, s, C--H).

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