Facile Preparation of Nucleoside-5′-carboxylic Acids

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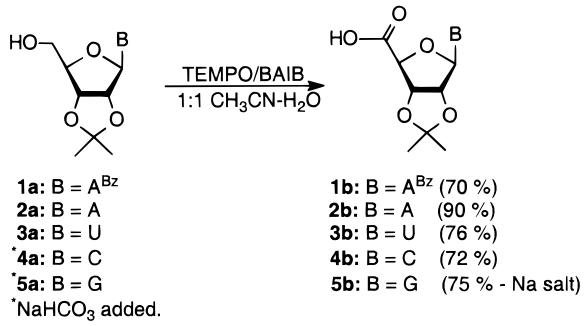
Oxidation of the 5′-hydroxymethylene of nucleosides to 5′-carboxylates is an essential step in the preparation of a number of biologically active molecules.[1] There are relatively few methods describing the general preparation of such nucleoside-5′-carboxylic acids. Herein, we report a mild procedure for the oxidation of 2′,3′-isopropylidene-protected purine- and pyrimidine-containing nucleosides to their respective 5′-carboxylic acids. This method gives high yields and has a very simple isolation procedure.

One of the most widely applied methods for affecting the oxidation of the 5′-hydroxyl of unprotected nucleosides employs molecular oxygen and a platinum catalyst.[2] However, this method affords relatively low yields when applied to 2′,3′-isopropylidene-protected nucleosides.[3] Instead, these nucleosides are most often oxidized using potassium permanganate under strongly alkaline reaction conditions.[4] This limits the potassium permanganate methodology to purine-containing nucleosides. Two other systems used to affect these conversions are CrO_3/acetic acid[5] and a two-step method involving the generation of the aldehyde followed by oxidation with *m*-CPBA.[6] However, these methods have not been generally applied.

Recently, a method utilizing ruthenium trichloride and sodium periodate under Sharpless conditions was used to obtain the 5′-carboxylic acids of 2′,3′-isopropylidenepurine-containing nucleosides in high yield.[7] Unfortunately, the methodology cannot be used with pyrimidine-containing nucleosides, as the reaction conditions cause loss of the nucleoside base. Extension of ruthenium trichloride-mediated oxidation to 2′,3′-isopropylidenepyrimidine-containing nucleosides requires the use of both alkaline conditions and potassium persulfate.[8] Thus, many available methods for the generation of nucleoside-5′-carboxylic acids require relatively basic conditions that limit their utility.

Scheme 1



1a: B = A^{Bz}
2a: B = A
3a: B = U
*__4a:__ B = C
*__5a:__ B = G
*$NaHCO_3$ added.

1b: B = A^{Bz} (70 %)
2b: B = A (90 %)
3b: B = U (76 %)
4b: B = C (72 %)
5b: B = G (75 % - Na salt)

A recent publication described the oxidation of alcohols to ketones and aldehydes using catalytic amounts of 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) and stoichiometric amounts of an organic oxidant, [bis(acetoxy)-iodo]benzene (BAIB).[9] The method drew our attention because of its mildness and efficiency. The active oxidant is an *N*-oxoammonium salt generated by dismutation of TEMPO; BAIB is necessary to regenerate TEMPO by oxidizing the corresponding hydroxylamine of TEMPO. The reaction generates acetic acid and iodobenzene as byproducts and is different from most other TEMPO-mediated oxidations in that it avoids inorganic salt contaminants.[9] In addition, *N*-oxoammonium salt-mediated oxidations are compatible with double and triple bonds, esters, ethers, acetals, epoxides, amides, halides, and azides. Finally, protecting groups such as TBDMS, THP, MOM, Boc, Cbz, benzyl, and acetyl are also stable to the reaction conditions.[10]

In the presence of high concentrations of water, *N*-oxoammonium salts convert aliphatic alcohols to their respective carboxylic acids.[10] Since the TEMPO−BAIB system seemed to be a particularly convenient method for *N*-oxoammonium salt oxidations, we hoped to obtain the desired 5′-carboxylates using the TEMPO−BAIB system. The original TEMPO−BAIB reference cited a single example of oxidation in an aqueous system, namely, the oxidation of an allylic alcohol to an allylic aldehyde in 1:1 acetonitrile−aqueous buffer (pH 7).[9] Since the presence of large amounts of buffer complicates the isolation of carboxylic acids in other TEMPO-mediated oxidations,[10] we hoped to utilize the TEMPO−BAIB oxidation system in a 1:1 acetonitrile−water solvent system without the use of large amounts of buffer (Scheme 1).

Indeed, in our first attempt, the 5′-carboxylic acid of 2′,3′-isopropylideneadenosine, **2b**, precipitated from the reaction solution. After 3 h of stirring at room temperature, the precipitated carboxylic acid was filtered and triturated sequentially with diethyl ether and acetone. This simple procedure yielded **2b** in 90% yield and high purity.[11] Similarly, the 5′-carboxylic acid of *N*-benzoyl-2′,3′-isopropylideneadenosine, **1b**, precipitated from solu-

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(11) The ^{1}H NMR data matched that previously reported except for the higher order coupling observed at 400 MHz. The ^{13}C NMR data also match previously reported frequencies, although the previously reported spectrum is offset by 3.9 ppm due to a referencing error.

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tion. Filtration and sequential trituration with diethyl ether and acetone gave **1b** in 70% yield and high purity.[12] We found that the diethyl ether wash removes the catalytic amounts of TEMPO and reaction byproducts and gives material that is sufficiently pure for all subsequent applications. However, trituration with acetone was necessary to obtain analytically pure samples. Since carboxylates **1b** and **2b** are essentially insoluble in acetone, the acetone trituration did not reduce the yields of these products. The ease of product isolation in these reactions makes the method ideal for large-scale preparations.

In contrast to the reaction behavior of the first two 5′-carboxylates, the 5′-carboxylate of 2′,3′-isopropylideneuridine, **3b,** oiled out of solution instead of precipitating as a solid. Consequently, after 3 h of stirring at room temperature, the reaction solution was concentrated to dryness, causing the product to solidify. The material was then triturated with diethyl ether and the product isolated by filtration. This alternative workup resulted in 76% yield of **3b** in good purity.[13,14] Thus, the catalytic amounts of TEMPO and reaction byproducts may be easily removed by diethyl ether trituration, even when the product does not crystallize from the reaction solution.

The extension of the methodology to 2′,3′-isopropylidene derivatives of cytidine and guanosine required some slight procedural modifications. Reaction of 2′,3′-isopropylidenecytidine under the previously stated conditions resulted in poor yields of the desired product. Since the starting material used was the hydrochloride salt, we added 1 equiv of sodium bicarbonate to achieve conditions similar to those used to obtain products **1b**−**3b**. Under these conditions, the desired 5′-carboxylic acid of 2′,3′-isopropylidenecytidine, **4b**, crystallized from the reaction solution. After filtration and trituration with diethyl ether, 30% of the expected **4b** was obtained in high purity.[15]

We were able to increase this yield by adding a second equivalent of sodium bicarbonate. With these modified reaction conditions, **4b** still precipitated from solution and was isolated in 57% yield, with no loss in product purity. Since the reaction of **4a** appears to be sensitive to the pH of the reaction solution, the second equivalent of sodium bicarbonate may be necessary to neutralize 1 equivt of the acetic acid generated as a reaction byproduct. Finally, since *N*-oxoammonium salts are known to be substantially more stable at 0 °C than at room temperature,[16] we sought a further increase in yield by carrying out the reaction at ice-bath temperatures. With this final modification, **4b** was produced in 72% yield and in high purity.

Initial attempts to obtain the 5′-carboxylic acid of 2′,3′-isopropylideneguanosine, **5b,** under the original reaction conditions used to obtain products **1b**−**3b** also resulted in poor yields. As in the case of 2′,3′-isopropylidenecytidine, the addition of an equivalent of sodium bicarbonate dramatically altered the reaction outcome. In the case of product **5b**, however, it was found that the sodium salt of the 5′-carboxylic acid precipitated from solution. This material was filtered and triturated sequentially with diethyl ether and acetone to yield 72% of **5b**. However, we found that the product was contaminated with a small amount of an impurity that could not be removed by simple trituration. In an attempt to remedy this, the reaction conditions were modified by adding a second equivalent of sodium bicarbonate. Under these modified reaction conditions, the sodium salt of the 5′-carboxylic acid was generated in 75% yield, free from the impurity generated under the previous reaction conditions.[17]

In summary, we have developed a mild, general procedure for the production of 5′-carboxylic acids of nucleosides. The method was used to generate the 5′-carboxylic acids of five 2′,3′-isopropylidene-protected nucleosides: adenosine, *N*-benzoyladenosine, uridine, cytidine, and guanosine. In all cases, the desired products were obtained in good yields and separated from TEMPO and reaction byproducts by trituration with diethyl ether. The mildness of this reaction and its tolerance of acid sensitive, base sensitive, and oxidatively labile functional groups should make it an attractive method for the oxidation of primary alcohols to carboxylic acids for both nucleosides and non-nucleosides. Finally, the method's facility and efficiency make it suitable for large-scale reactions.

Experimental Section

General. Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 400 and 100.6 MHz, respectively. The chemical shifts are expressed in parts per million (δ) relative to tetramethylsilane for ^{1}H NMR and relative to DMSO-d_6 for ^{13}C NMR. Chemicals 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO), bis(acetoxy)iodo]benzene (BAIB), 2′,3′-isopropylideneuridine, and 2′,3′-isopropylideneadenosine were purchased from Aldrich Chemical Co; 2′,3′-isopropylidenecytidine (hydrochloride salt) and 2′,3′-isopropylideneguanosine were purchased from Sigma Chemical Co. Finally, *N*-benzoyl-2′,3′-isopropylideneadenosine was obtained by the method of Chladek and Smrt.[18]

General Procedure for Generation of Nucleoside-5′-carboxylates. BAIB (709 mg, 2.2 mmol), TEMPO (32 mg, 0.2 mmol), and a 2′,3′-isopropylidene-protected nucleoside (1 mmol) were combined in a reaction vessel, and to this mixture was added 2 mL of a 1:1 acetonitrile−water solution. The reaction mixtures were stirred for 3 h before the respective products were isolated as individually described below.

***N*^{6}-Benzoyl-2′,3′-isoproylideneadenosine-5′-carboxylic Acid (1b).** The resulting precipitate was filtered, triturated sequentially with diethyl ether and acetone, and dried in vacuo. Yield: 70%. Mp: 208−209 °C. ^{1}H NMR (DMSO-d_6, 50 °C): δ 11.0 (1H, br s, NH); 8.68 and 8.64 (2 × 1H, 2 × s, H-2 and H-8); 8.04, 7.62 and 7.54 (5H, m, Ph); 6.45 (1H, s, H-1′); 5.54 (1H, d, J = 6.0 Hz, H-2′); 5.51 (1H, dd, J = 6.0 and 1.7 Hz, H-3′); 4.72 (1H, d, J = 1.7 Hz, H-4′); 1.55 and 1.38 (2 × 3H, 2 × s, CMe_2). ^{13}C NMR (DMSO-d_6, 20 °C): δ 170.61, 165.55, 151.93, 151.18, 150.12, 143.85, 133.43, 132.21, 128.32, 128.28, 125.25, 112.68, 89.91, 85.74, 83.7, 83.47, 26.45, 24.89. Anal. Calcd for $C_{20}H_{19}N_5O_6$: C, 56.47; H, 4.50; N, 16.46. Found: C, 56.25; H, 4.59; N, 16.38.

(12) Although we report the ^{1}H and ^{13}C NMR's in DMSO-d_6, a ^{1}H NMR spectra of the product in pyridine-d_5 was consistent with a previous report (see ref 6).

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(14) Although the material was of good purity, recrystallization from acetone was necessary to obtain an analytically pure sample. The ^{1}H NMR of the product was spectroscopically identical to previous 60 MHz data, except for the higher order coupling observed at 400 MHz.

(15) Although there was a report of this compound in the literature, it appears that we are the first to report physical data for this compound (see ref 8).

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(17) Although the material was of good purity, recrystallization from acetone−water was necessary to obtain an analytically pure sample. To the best of our knowledge, there are no prior reports of this compound in the literature. The ^{1}H and ^{13}C NMR spectra of the isolated product are in good agreement with the proposed structure.

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2′,3′-*O*-Isoproylideneadenosine-5′-carboxylic Acid (2b). The resulting precipitate was filtered, triturated sequentially with diethyl ether and acetone, and dried in vacuo. Yield: 90%. Mp: 246−249 °C dec. ^{1}H NMR (DMSO-d_6, 50 °C): δ 8.23 and 8.10 (2 × 1H, 2 × s, H-2 and H-8); 7.15 (2H, s, NH_2); 6.32 (1H, s, H-1′); 5.52 (1H, dd, J = 6.0 and 1.9 Hz, H-3′); 5.48 (1H, d, J = 6.0 Hz, H-2′); 4.67 (1H, d, J = 1.9 Hz, H-4′); 1.53 and 1.36 (2 × 3H, 2 × s, CMe_2). ^{13}C NMR (DMSO-d_6, 50 °C): δ 170.36, 155.84, 152.15, 149.03, 140.11, 118.70, 112.66, 89.48, 85.14, 83.52, 83.27, 26.39, 24.86. Anal. Calcd for $C_{13}H_{15}N_5O_5$: C, 48.60; H, 4.71; N, 21.80. Found: C, 48.43; H, 4.77; N, 21.62.

2′,3′-*O*-Isoproylideneuridine-5′-carboxylic Acid (3b). The product separated from the reaction solution as an oil. Therefore, the solvents were removed in vacuo, and the resulting residue was triturated with diethyl ether, filtered, and dried in vacuo. Yield: 76%. An analytically pure sample was obtained by recrystallization from acetone. Mp: 233−235 °C. ^{1}H NMR (DMSO-d_6, 50 °C): δ 12.6 (1H, br s, COOH); 11.22 (1H, br s, NH); 7.76 (1H, d, J = 7.9 Hz, H-6); 5.77 (1H, s, H-1′); 5.59 (1H, dd, J = 7.9 and 2.2 Hz, H-5); 5.20 (1H, dd, J = 6.2 and 2.1 Hz, H-3′); 5.15 (1H, d, J = 6.2 Hz, H-2′); 4.54 (1H, d, J = 2.1 Hz, H-4′); 1.46 and 1.31 (2 × 3H, 2 × s, CMe_2). ^{13}C NMR (DMSO-d_6, 50 °C): δ 170.50, 163.14, 150.57, 144.23, 112.11, 101.24, 95.29, 86.45, 83.80, 83.52, 26.33, 24.69. Anal. Calcd for $C_{12}H_{14}N_2O_7$: C, 48.33; H, 4.73; N, 9.39. Found: C, 48.12; H, 4.74; N, 9.43.

2′,3′-*O*-Isoproylidenecytidine-5′-carboxylic Acid (4b). Two equivalents of sodium bicarbonate were added to the reagents listed in the general procedure. The reaction was conducted at ice-bath temperature for the first 2 h and then allowed to warm to room temperature over the third hour. The resulting precipitate was filtered, triturated sequentially with diethyl ether and acetone, and dried in vacuo. Yield: 72%. Mp: 244−246 °C dec. ^{1}H NMR (DMSO-d_6, 20 °C): δ 7.78 (1H, d, J = 7.4 Hz, H-6); 7.34 (2H, br d, NH_2); 5.70 (1H, d, J = 7.4 Hz, H-5); 5.65 (1H, s, H-1′); 5.24 (1H, dd, J = 6.0 and 2.0 Hz, H-3′); 5.05 (1H, d, J = 6.2 Hz, H-2′); 4.48 (1H, d, J = 1.9 Hz, H-4′); 1.45 and 1.29 (2 × 3H, 2 × s, CMe_2). ^{13}C NMR (DMSO-d_6, 50 °C): δ 170.71, 165.96, 155.03, 144.64, 111.90, 96.04, 93.75, 86.53, 84.09, 83.91, 26.45, 24.73. Anal. Calcd for $C_{12}H_{15}N_3O_6$: C, 48.49; H, 5.09; N, 14.14. Found: C, 48.49; H, 5.10; N, 14.07.

2′,3′-*O*-Isoproylideneguanosine-5′-carboxylic Acid (5b). Two equivalents of sodium bicarbonate were added to the reagents listed in the general procedure. The resulting precipitate was filtered, triturated sequentially with diethyl ether and acetone, and dried in vacuo. Yield: 75%. An analytically pure sample was obtained by recrystallization from acetone−water. Mp: 246−248 °C dec. ^{1}H NMR (DMSO, 50 °C): δ 11.04 (1H, br s, N−H); 8.32 (1H, s, H-8); 6.65 (2H, s, NH_2); 5.95 (1H, d, J = 2.6 Hz, H-1′); 5.06 (1H, dd, J = 6.0 and 1.7 Hz, H-3′); 4.95 (1H, dd, J = 6.0 and 2.1 Hz, H-2′); 4.32 (1H, d, J = 1.7 Hz, H-4′); 1.50 and 1.30 (2 × 3H, 2 × s, CMe_2). ^{13}C NMR (DMSO, 50 °C): δ 172.38, 157.33, 154.13, 150.83, 136.29, 116.16, 112.21, 89.22, 87.09, 84.55, 83.80, 26.87, 25.03. Anal. Calcd for $C_{13}H_{14}N_5NaO_6 \cdot 2H_2O$: C, 39.5; H, 4.59; N, 17.72; Na, 5.82. Found: C, 39.64; H, 4.58; N, 17.37; Na, 6.09.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1b**−**5b** (10 pages). See any current masthead page for ordering and Internet access information.

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