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Uracil ring opening in the reaction of 5-formyl-2′-deoxyuridine with primary alkyl amines

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Abstract—Treatment of 5-formyl-2′-deoxyuridine (f^5dU) with stoichiometric amounts of strongly nucleophilic, non-hindered primary alkyl amines led to fast and quantitative formation of the corresponding Schiff bases. In the presence of excess amines, novel nucleosides with ring opened pyrimidine bases were formed as a result of the Michael addition of a second amine to the pre-formed imines. In the reaction of f^5dU with aromatic amines, the formation of Schiff base derivatives was slower and even under prolonged treatment with an excess of amine the uracil ring remained intact.

5-Formyl-2′-deoxyuridine (f^5dU) is a base-modified nucleoside containing a reactive electrophilic aldehyde group. The reactivity of the 5-formyl nucleoside towards nucleophilic amine components is important for its biological properties in cells,[1–5] as well as for the application of f^5dU in the chemical synthesis of 5-amino modified 2′-deoxyuridines.[6–12]

5-Formyl-2′-deoxyuridine is a well known oxidative thymine lesion in DNA which exhibits significant cytotoxicity and mutagenicity.[1–4] It has been suggested that the formyl group of f^5dU may react with amino groups of DNA-binding proteins (mainly with the lysine ε-NH_2 group) to form potentially lethal covalent cross linked imines.[1,3,5] On the other hand, in model studies, no Schiff base products were observed (NMR and UV data) in the reaction of a free 5-formyl-2′-deoxynucleoside with primary amines and amino acids in neutral aqueous solution simulating physiological conditions.[5]

5-Formyl-2′-deoxyuridine has been used as a substrate for the synthesis of 5-amino modified nucleosides via reductive amination.[6–12] In reported experiments of f^5dU reductive amination, the intermediate imine derivatives were neither isolated nor characterized. Instead, the Schiff bases were converted in situ by subsequent reduction into the stable amine derivatives.

Various 5-amino modified pyrimidine nucleosides have found wide application as useful units for the modification of nucleic acids, including labeling[13] and structure stabilization[14] as well as for the introduction of additional functionality to nucleic acids designed as aptamers, biosensors or catalysts.[15,16] In particular, pyrimidine nucleosides modified with functional groups that mimic the side chain of amino acids were used successfully for the selection of new deoxyribozymes.[17–19]

As a part of our recent studies on the structure–function relationships of deoxyribozyme '10–23',[20,21] we were interested in the reductive amination of 5-formyl-2′-deoxyuridine as a method for the preparation of 2′-deoxyuridines bearing 'protein-like' modifications at the 5-position of the base moiety. Our initial attempts to obtain 2′-deoxyuridine modified with a histamine residue via reductive amination[22] afforded the desired 5-histaminylmethyl-2′-deoxyuridine in low yield >15%. We also observed that a higher molar excess of histamine compared to aldehyde (five-fold) and extended reaction time for intermediate imine formation (up to 24 h) led to a complicated mixture of products.

To explain the above observations we undertook detailed model studies on the first step of the reductive amination reaction, namely, the formation of Schiff

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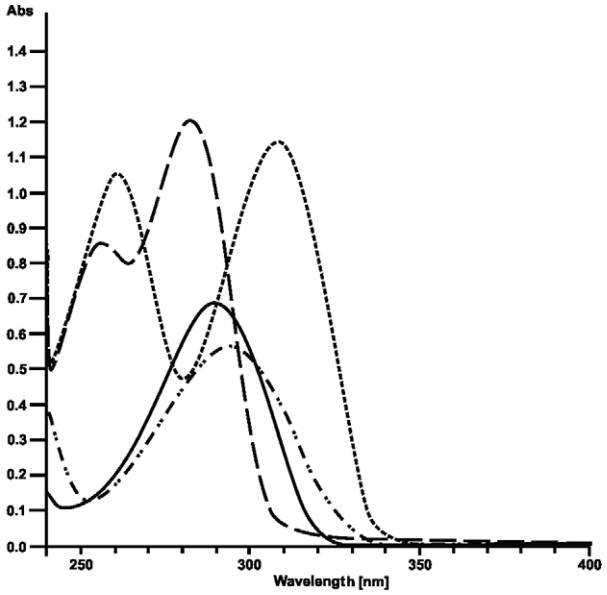
bases in the reaction of 5-formyl-2′-deoxyuridine and the selected primary amines.

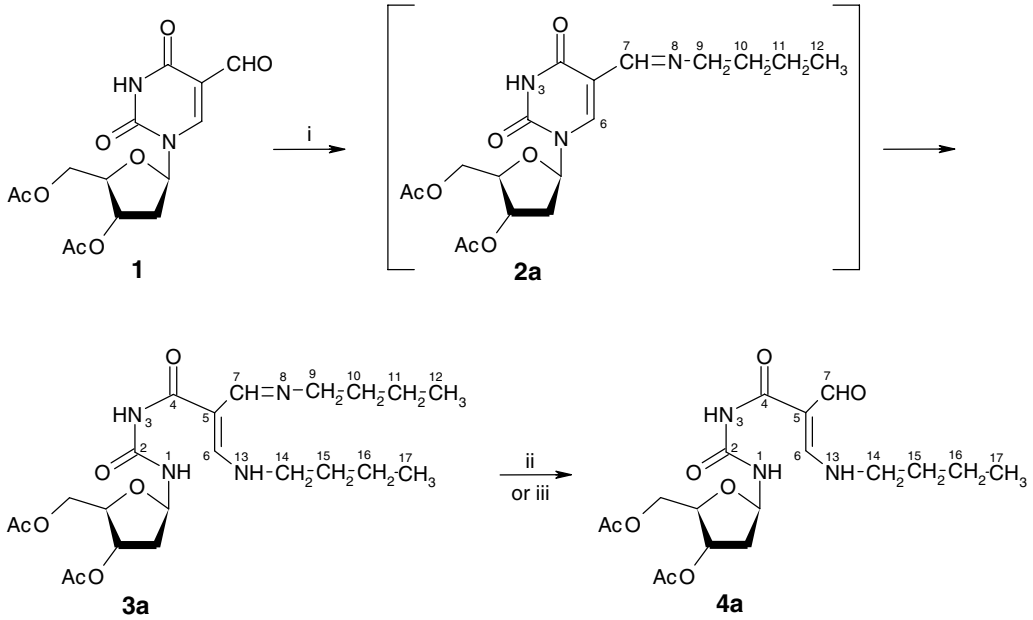
We started our experiments using 5-formyl-2′-deoxyuridine protected with acetyl groups at the sugar moiety[10] and *n*-butylamine as a model amine with nucleophilicity close to that of the primary amine function of histamine ($pK_a = 10.7$ and 10.6, respectively). Initially, aldehyde **1** was reacted with five equivalents of *n*-butylamine in anhydrous CH_2Cl_2 at room temperature (Scheme 1). TLC analysis (silica gel, $CHCl_3$/MeOH, 9/1 v/v) revealed the formation of one product with higher chromatographic mobility as compared to **1** ($R_f = 0.73$ for the new product, $R_f = 0.47$ for **1**).

A ^1H NMR spectrum of the crude reaction mixture[23] after 24 h showed complete disappearance of the C*H*O signal at δ_H 10.03. A single product was isolated from the reaction mixture by silica gel column chromatography (78%, elution with ethyl acetate). Spectral analysis of this product clearly indicated that instead of the expected Schiff base derivative **2a**, adduct **3a** containing two *n*-butylamine residues was formed (Scheme 1). This result can be explained by nucleophilic attack of one amine molecule at the 5-formyl function of **1** followed by Michael type addition of the second amine to the C5=C6 double bond with subsequent opening of the uracil ring. The structure of **3a** was confirmed by FAB MS, ^1H and ^{13}C NMR[24] data (see Supplementary data) as well as by the UV spectrum (Fig. 1). In the FAB MS, the measured *m*/*z* values for $[M+H]^+$ and $[M-H]^-$ were consistent with the MW calculated for **3a**. The UV spectrum ($CHCl_3$) of **3a** indicated changes in the structure of the heterobase moiety as compared to the structure of the starting f^{5}dU (Fig. 1). In the ^1H NMR spectrum, three deuterium exchangeable signals for protons bound to nitrogen atoms: N3 (δ_H 13.04, br s), N13 (δ_H 9.36, m) and N1 (δ_H 9.13, d), a singlet due to the C7 proton and a doublet for the C6 proton (which collapsed to a singlet upon N(13)H deuteration) clearly supported the structure of uracil ring-opened product **3a**. It is worth noting that although adduct **3a** can exist in many isomeric and



derivative	line	λ_{max} (ε)	λ_{min}	λ_{max} (ε)	λ_{min}
1	———	291 (14000)	241	-	-
2a	– ·· –	296 (11200)	253	-	-
3a	-------	309 (22700)	281	262 (20800)	242
4a	– – –	284 (24800)	265	252 (17500)	242

Figure 1. UV spectra of **1** and **2a**–**4a** in $CHCl_3$, $c = 5.0 \times 10^{-5}$ mol/dm^3.



Scheme 1. The reaction of 3′,5′-di-*O*-acetyl-5-formyl-2′-deoxyuridine with *n*-butylamine. Reagents and conditions: (i) *n*-butylamine (5 equiv), CH_2Cl_2, rt, 24 h; (ii) CH_3COOH (10 equiv), CH_2Cl_2:H_2O, 1:1 v/v, rt, 48 h; (iii) MeOH:H_2O, 1:1 v/v, rt.

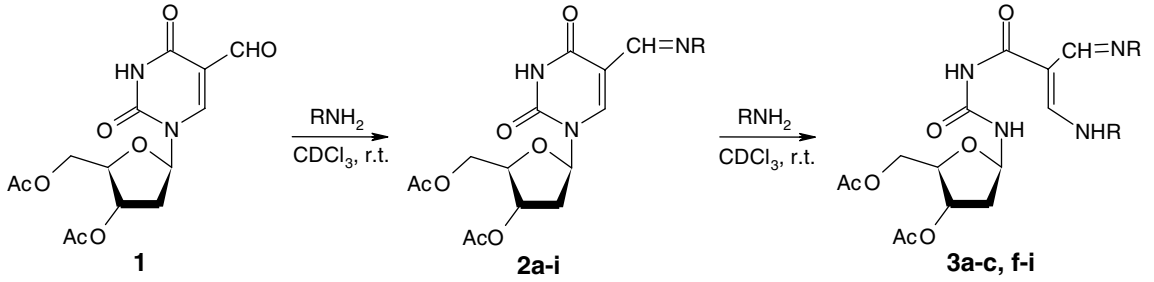
tautomeric forms, only one was observed in $CDCl_3$, probably due to stabilization of the structure by intramolecular hydrogen bonds. This assumption is supported by the ^1H NMR data of **3a** in CD_3OD. The presence of one broad singlet at δ 7.49 for the protons at C6 and C7 (see Supplementary data) indicates dynamic exchange of tautomers in protic conditions. More detailed NMR studies on the structure of **3a** are in progress.

Although adduct **3a** is relatively stable in organic solutions, its prolonged storage in wet organic solvents resulted in decomposition. Partial hydrolysis of the imine function of **3a**, leading to compound **4a**, was observed in chloroform containing traces of water. In $MeOH/H_2O$ solution, deprotection of the acetyl groups from the sugar moiety of **4a** was observed due to the action of *n*-butylamine liberated during hydrolysis of the imine function. Pure **4a** was isolated in 90% yield after treatment of **3a** with 10 equiv of acetic acid for 48 h at room temperature (Scheme 1). The structure of **4a** was confirmed by FAB MS, ^1H and ^{13}C NMR analysis.[25] The differences in the chromophore systems of **3a** and **4a** are clearly seen in their UV spectra (Fig. 1).

A compound of type **4a** has previously been described by Catalanotti and co-workers.[9] It was isolated as a by-product of the direct (one-pot) reductive amination of 3′,5′-*O*-(tetraisopropyldisiloxane-1,3-diyl)-2′-deoxy-5-formyluridine with twenty molar equivalents of *n*-butylamine and $NaBH_3CN$ as the reducing agent in THF or DMF. The authors[9] claimed that the observed opening of the pyrimidine ring was promoted by $NaBH_3CN$ in polar solvents. Since we observed the formation of **4a** without assistance of any reducing agent, we suggest that this product is formed exclusively by hydrolysis of adduct **3a**.

In order to determine whether adduct **3a** was the product of subsequent addition of a second amine molecule to the already formed Schiff base **2a**, the reaction of aldehyde **1** with *n*-butylamine at different molar ratios was performed in anhydrous $CDCl_3$ and the progress of the reaction was monitored by ^1H NMR spectroscopy (Table 1, entry a). A spectrum taken 5 min after mixing stoichiometric amounts of the reagents showed quantitative formation of Schiff base **2a**. The characteristic proton signal for the aldehyde disappeared and a new singlet corresponding to the azomethine proton appeared close to the C(6)H singlet (signals at δ_H 8.30 and 8.28). The structure of **2a** was confirmed by UV (Fig. 1) and FAB MS analysis (see Supplementary data). Spectral monitoring of the reaction of **1** with 5 equiv of *n*-butylamine revealed that besides the peaks corresponding to the Schiff base **2a**, new signals characteristic for adduct **3a** also appeared, and after 1 h, **3a** was present in ca. 20%. After 24 h, **3a** was the major product in the reaction mixture.

Table 1. Formation of Schiff bases **2** and diadducts **3** in the reaction of 5′,3′-di-*O*-acetyl-5-formyl-2′-deoxyuridine with primary amines **a**–**i** monitored by ^1H NMR spectroscopy



Entry	Amine	% of Schiff base **2**[a]		% of diadduct **3**	
		1.0 equiv of amine	5.0 equiv of amine	5.0 equiv of amine	
		5 min	5 min	1 h	24 h
a	*n*-Butylamine	100	100	20	85
b	*t*-Butylamine	45[b]	80	<5	25[d]
c	Benzylamine	100	100	5	30[e]
d	Aniline	35[c]	100	0	0
e	*p*-Toluidine	35	60	0	0
f	Ethanolamine	100	100	30	90
g	Ethylene diamine	100	100	20	80
h	Putrescine	100	100	30	95
i	Histamine[f]	85	100	20	80

[a] Yields were determined from the ^1H NMR spectra as the integral ratio of the signals of the anomeric and C(6) protons of products **2** and **3** present in the reaction mixtures.
[b] 60% of Schiff base **2b** after 24 h.
[c] 90% of Schiff base **2d** after 24 h.
[d] ~50% of diadduct **3b** after 11 d.
[e] 95% of diadduct **3c** after 11 d.
[f] The histamine derivative with MMTr on the imidazole ring,[26] liberated in situ from its trifluoroacetate by treatment with triethylamine, was used as the amine component.

To explore the scope of the reaction of f^5dU with primary amines, we investigated this reaction with selected amines (Table 1) of varying nucleophilicity (aliphatic and aromatic amines), steric hindrance (*t*-butylamine) and with additional functionality present (e.g., putrescine and histamine). In all the ^1H NMR spectra recorded after 5 min reaction of equimolar amounts of amines and aldehyde **1**, proton signals indicating exclusive formation of the Schiff base products **2** were observed. However, for sterically hindered *t*-butylamine (Table 1, entry b) and for weak nucleophilic aromatic amines (entries d and e) the yields of compound **2** were not quantitative. When five molar equivalents of *t*-butylamine or *p*-toluidine were used, after 5 min, substrate **1** was still present in the reaction mixtures (Table 1, entries b and e). In similar experiments with *n*-butylamine, ethanolamine, ethylene diamine, putrescine and histamine, besides peaks due to Schiff base derivatives **2**, low intensity signals (~5%) consistent with the structure of adducts **3** started to appear. When the reactions with an excess of amine were left for 24 h, the respective ring-opened compounds **3a**, **f–i** were the main products (Table 1, entries a, f–i). In the case of the reaction of **1** with hindered *t*-butylamine, adduct **3b** was formed in 25% yield only. The Michael type addition of a second amine molecule with subsequent opening of the uracil ring was also slower with benzylamine. In this case, the reaction was complete in eleven days (entry c). No products of type **3** were obtained in the reactions of **1** with excess aromatic amines (entries d and e). In these experiments, the Schiff base derivatives **2d** and **2e** were the only products formed, as observed by NMR spectroscopy even after 11 days.

In summary, we have found that transformation of 5-formyl-2′-deoxyuridine **1** to the corresponding Schiff base strongly depends on the nature of the amine and the reaction conditions, particularly, the amine excess over f^5dU. In the experiments performed in anhydrous methylene chloride or chloroform with stoichiometric amounts of the strongly nucleophilic, non-hindered primary alkyl amines, the corresponding imines were formed rapidly and quantitatively. With excess amines (5 equiv), subsequent Michael addition occurred leading to uracil ring opening and formation of products **3**. Reactions of **1** with aromatic amines of a weak nucleophilicity were slower and the resulting Schiff bases **2** did not undergo subsequent Michael addition even in excess of the amine component was used and the reaction time was significantly prolonged.

Our results provide new insight into the process of the formation of Schiff bases derived from 5-formyl-2′-deoxyuridine and their susceptibility for subsequent transformations. The obtained results may be helpful in the design and optimization of 5-amino modified 2′-deoxyuridine synthesis via stepwise or one-pot reductive amination.

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Supplementary data

Supplementary data associated with this article contain spectral data for **2a**, **3a** and **4a** (FAB MS, ^1H and ^{13}C NMR). Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.12.113.

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22. The reaction of 5′,3′-di-*O*-acetyl-5-formyl-2′-deoxyuridine with the histamine derivative protected with an MMTr group on the imidazole ring (2 molar equivalents) was performed in anhydrous CH_2Cl_2 for 3 h, followed by imine reduction with $NaBH_3CN$ at room temperature.
23. The excess *n*-butylamine was removed from the reaction mixture by repeated co-evaporation with anhydrous toluene and the residue was dissolved in anhydrous $CDCl_3$.

24. Spectral data for diadduct **3a**: FAB HRMS *m/z* calculated for $[M-H]^-$ $C_{22}H_{35}N_4O_7$ 467.2506, found 467.2497; ^{1}H NMR ($CDCl_3$; 250 MHz) δ (ppm), *J* in Hz: 0.94 (t, 6H, $^3J = 7.3$ C(12)H_3 and C(17)H_3); 1.35–1.40 (m, 4H, –C(11)H_2 and –C(16)H_2); 1.54–1.68 (m, 4H, –C(10)H_2 and –C(15)H_2); 2.08 (s, 3H, CH_3COO); 2.13 (s, 3H, CH_3COO); 2.14 (m, 1H, H2′); 2.23 (ddd, 1H, $^3J_{2''3'} = 2.0$, $^3J_{2''1'} = 5.7$, $^2J_{2''2'} = -14.0$, H2″); 3.29–3.38 (m, 4H, –C(9)H_2 and –C(14)H_2); 4.14–4.24 (m, 3H, H4′, H5′, H5″); 5.18 (m, 1H, H3′); 6.01 (ddd, 1H, $^3J_{1'2''} = 5.7$, $^3J_{1'2'} = 8.4$, $^3J_{H1'N(1)H} = 9.3$ Hz, H1′); 6.92 (d, 1H, $^3J_{C(6)HN(13)H} = 13.4$, C(6)H); 7.57 (s, 1H, C(7)H); 9.13 (d, 1H, $^3J_{N(1)HH1'} = 9.3$ N(1)H); 9.36 (m, 1H, –N(13)H); 13.04 (br s, 1H, N(3)H); ^{13}C NMR ($CDCl_3$; 63 MHz) δ (ppm): 13.18, 13.47 (C-12, C-17); 19.21, 19.93 (C-11, C-16); 20.36, 20.52 ($2 \times CH_3$ from CH_3COO); 32.38, 33.03 (C-10, C-15); 37.50 (C-2′); 49.04 (C-14); 59.45 (C-9); 64.11 (C-5′); 74.79 (C-3′); 80.50 (C-4′); 80.84 (C-1′); 95.89 (C-5); 153.80 (C-2); 159.36, 159.90 (C-6, C-7); 169.31 (C-4); 170.05, 170.18 ($2 \times$ CO from CH_3COO).

25. Spectral data for **4a**: HRMS *m/z* calculated for $[M-H]^-$ $C_{18}H_{26}N_3O_8$ 412.1720, found 412.1709; ^{1}H NMR ($CDCl_3$; 250 MHz) δ (ppm), *J* in Hz: 0.99 (t, 3H, $^3J = 7.3$, C(17)H_3); 1.33–1.54 (m, 2H, –C(16)H_2); 1.76–1.57 (m, 2H, –C(15)H_2); 2.08 (s, 3H, CH_3COO); 2.12 (s, 3H, CH_3COO); 2.14 (m, 1H, H2′); 2.34 (ddd, 1H, $^3J_{2''3'} = 2.0$, $^3J_{2''1'} = 5.7$, $^3J_{2''2'} = -14.0$, H2″); 3.45 (q, 2H, $^3J = 6.6$, –C(14)H_2); 4.07–4.31 (m, 3H, H4′, H5′, H5″); 5.18 (dt, 1H, $^3J_{3'2'} = 6.1$, $^3J_{3'2''} = {}^3J_{3'4'} = 2.0$, H3′); 6.01 (ddd, 1H, $^3J_{1'2''} = 5.9$, $^3J_{1'2'} = 8.8$, $^3J_{1'NH1} = 9.0$, H1′); 7.33 (d, 1H, $^3J_{C(6)HN(13)H} = 14.1$, C(6)H); 8.89 (d, 1H, $^3J_{N(1)HH1'} = 9.0$ N(1)H); 8.98 (s, 1H, C(7)H); 9.96 (m, 1H, N(13)H); 11.05 (s, 1H, N(3)H); ^{13}C NMR ($CDCl_3$; 63 MHz) δ (ppm): 13.40 (C-17); 19.47 (C-16); 20.68, 20.83 ($2 \times CH_3$ from CH_3COO); 32.09 (C-15); 37.77 (C-2′); 50.29 (C-14); 64.35 (C-5′); 74.85 (C-3′); 80.80 (C-4′); 81.10 (C-1′); 101.82 (C-5); 152.99 (C-2); 165.42 (C-6); 168.06 (C-4); 170.38, 170.49 ($2 \times$ CO from CH_3COO); 187.46 (C-7).

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