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Photochemical reactions of 5-fluoropyrimidine bases with selected alkylamines

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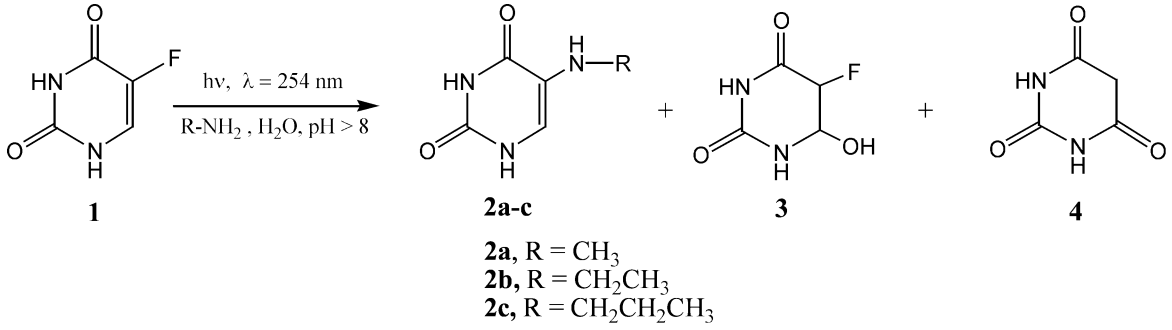
Abstract—Novel photochemical reactions between 5-fluoropyrimidine bases and primary alkylamines are described. Photoreaction of 5-fluorouracil with alkylamines in aqueous solution (pH>8) leads to 5-alkylaminouracils as the main photoproducts. Similarly, photoreaction of 5-fluorocytosine with alkylamines yields 5-alkylaminocytosines.

The photochemistry of 5-halogenopyrimidine bases has been extensively studied because of the enhanced photoreactivity of 5-halogenopyrimidine-substituted nucleic acids as compared to native nucleic acids and the potential use of photochemical cross-linking of such nucleic acids with proteins to investigate contact positions located in nucleoprotein complexes.[1] Despite the fact that there is ample literature on photocross-linking of proteins to 5-halogenopyrimidine substituted nucleic acids, detailed information on the chemical structures of the conjugates and the reaction mechanisms involved in their formation is often lacking.[2,3] In this study, we have focused on the amino group functionality which is present in the side-chain of the amino acid lysine and *N*-terminal amino acid residue of proteins. We report here our results on the isolation and characterization of products obtained from photochemical reactions of 5-fluorouracil **1** and 5-fluorocytosine **8** with primary alkylamines.

Irradiation of 5-fluorouracil **1** and the appropriate alkylamine with 254 nm ultraviolet light in aqueous solution (pH>8) yields 5-alkylaminouracils **2a–c** and by-product 5-fluoro-6-hydroxy-5,6-dihydrouracil **3** which partially undergoes conversion to barbituric acid **4** by elimination of hydrogen fluoride (Scheme 1).[4] Formation of small amounts of some uncharacterized minor products was also detected by HPLC.

The structures of the products **2a–c** were established on the basis of spectroscopic data (UV, NMR and MS)[5] as well as by independent syntheses from 5-bromouracil and alkylamines (thermal reactions).[6] In addition, we have also synthesized 6-alkylaminouracils[7] by thermal reaction of 6-chlorouracil and alkylamines and used them in identification procedures in order to exclude the possibility that these compounds are the products of our photoreactions.



1 **2a-c** **3** **4**

2a, R = CH_3
2b, R = CH_2CH_3
2c, R = $CH_2CH_2CH_3$

Scheme 1.

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The photoreactions were monitored by high performance liquid chromatography (HPLC) using a photodiode array detector (PAD) and a mass detector (electrospray ionisation mass spectrometry, ESI MS).[8] We have detected the major product **2** by HPLC during the early stages of the photoreaction. It was not possible to observe postulated precursors of **2** (Scheme 2), which should have UV spectra lacking an absorption band with a maximum at about 270 nm, which is characteristic of the pyrimidine chromophore with a saturated double bond at C(5)=C(6). This suggests that the transformation of 5-fluoro-6-alkylamino-5,6-dihydrouracil **5** (Scheme 2) is a process occurring on a faster time scale than that of HPLC experiments designed to detect it.

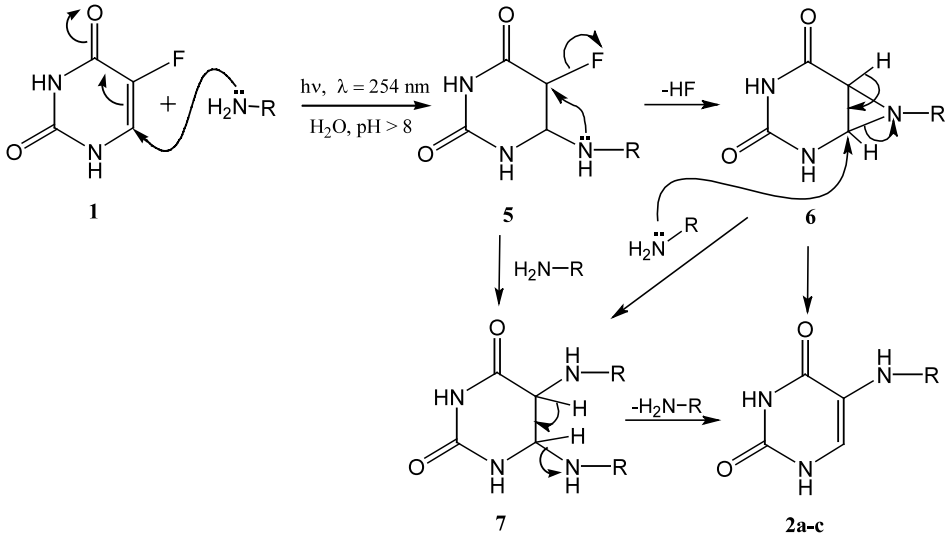
Treatment of pure photohydrate **3** with an alkylamine does not produce **2**. This experiment allowed us to exclude the possibility that product **2** is formed in a dark reaction via **3**. Irradiation of **3** and an alkylamine with 254 nm ultraviolet light in aqueous solution likewise does not yield **2** (on the other hand **3** does not absorb light significantly at 254 nm); this eliminates the possibility that **2** is formed by a photochemical reaction from **3**. It is reasonable to postulate that the first step of the reaction involves nucleophilic attack of an amine on the C-6 position of excited 5-fluorouracil to give the intermediate product **5**. It can be noted that, other nucleophiles (e.g. water[9] and alcohols[10]) attack the C-6 position of excited 5-fluoropyrimidine bases. Subsequently, the intermediate **5** could undergo nucleophilic displacement of the fluorine substituent at the 5 position either by an amine acting as external nucleophile to yield **7** or intramolecularly with the aid the of 6-alkylamino group (neighboring group effect) to produce the aziridine intermediate **6**.

It is interesting to note that photoreactions of 5-bromouracil with alkylamines in aqueous solution yield mainly uracil (debromination reaction) and, in addition, ring opened products namely *N*-(*N*′-alkylcarbamoyl)-3-amino-2-bromoacrylamides.[11] In the first case, cleavage of C(5)–Br bond takes place (which is much weaker than C(5)–F bond), in the second, nucleophilic attack of an amine on the C-2 position of the pyrimidine ring of 5-bromouracil causes the splitting of the C(2)–N(1) bond and formation of the ring opened product. The photoreactions of non-halogenated pyrimidine bases (uracil,[12] thymine,[13] cytosine[12,14] and 5-methylcytosine[14]) with alkylamines yield mainly ring opened products, which subsequently undergo thermal ring closure to 1-alkylpyrimidine bases.

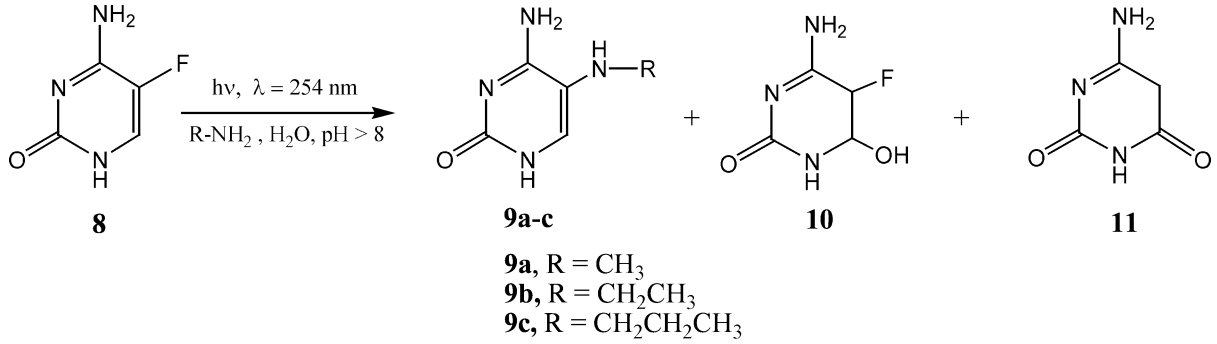
Analogously, irradiation of 5-fluorocytosine **8** and the appropriate alkylamine with 254 nm ultraviolet light in aqueous solution (pH>8) produces 5-alkylaminocytosines **9a**–**c** and by-product 5-fluoro-6-hydroxy-5,6-dihydrocytosine **10** which undergoes partial transformation to 4-amino-2,6-dihydroxypyrimidine **11** by elimination of hydrogen fluoride (Scheme 3).[4] Formation of some unidentified minor products was also detected by HPLC.

The identity of products **9a**–**c** was established by comparison of their spectral data[15] with those obtained from thermal reactions of 5-bromocytosine and alkylamines at 90–100°C. The photochemical reactions of 5-fluorocytosine with alkylamines presumably proceed by a similar mechanism to that proposed in Scheme 2 above for 5-fluorouracil and alkylamines. Because the photoreactions of 5-fluorouracil and 5-fluorocytosine with alkylamines are not photosensitized by acetone and as the presence of oxygen has no significant effect on product formation, it can be concluded that they proceed with involvement of a singlet excited state of 5-fluoropyrimidine bases.

In conclusion, we have shown that photochemical reaction of 5-fluoropyrimidine bases with alkylamines yields 5-alkylaminopyrimidines as the major products. This suggests possible modes of photoinduced cross-linking between the amine side chain of the amino acid lysine



Scheme 2.



Scheme 3.

and the *N*-terminal amino acid residue contained in proteins and 5-fluoropyrimidine-substituted nucleic acids.

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4. General procedure for irradiations: An aqueous solution of 5-fluoropyrimidine base (0.2 mmol) and appropriate alkylamine (200 mmol) at pH>8 (pH was usually adjusted to 9 with hydrochloric acid) was irradiated for 4 h in a cylindrical reactor using an immersed, water-cooled, 15 W low pressure mercury lamp (Heraeus, Germany) with a cylindrical Vycor light filter (2 mm thick). After removal of solvent in vacuo, the products **2a–c** and **9a–c** were isolated by preparative HPLC on XTerra Prep RP_{18} reverse phase column (19×100 mm, 5 μm) using water–methanol (90:10 v/v) as eluent in yields 36–40%.
5. Compound **2a**: UV (in 20 mM aqueous KH_2PO_4 pH 2.5): $\lambda_{max}=262$ nm. EIMS (high resolution): calcd for $C_5H_7N_3O_2$ 141.05302, found 141.05383. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.25 (s, 3H, CH_3), 4.23 (s, 1H, C^5-NH), 6.19 (s, 1H, H-6), 10.15 (s, 1H, N-1), 11.10 (s, 1H, N-3). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 39.5, 111.3, 125.2, 149.2, 161.0. Compound **2b**: UV (in 20 mM aqueous KH_2PO_4 pH 2.5): $\lambda_{max}=261$ nm. EIMS (high resolution) calcd for $C_6H_9N_3O_2$ 155.07054, found 155.06947. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.10 (t, $J=7.3$ Hz, 3H, CH_3), 2.80 (q, $J=7.3$ Hz, 2H, CH_2), 4.10 (s, 1H, C^5-NH), 6.15 (s, 1H, H-6), 10.16 (s, 1H, N-1), 11.13 (s, 1H, N-3). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 13.9, 40.3, 112.4, 132.9, 149.4, 161.3. Compound **2c**: UV (in 20 mM aqueous KH_2PO_4 pH 2.5): $\lambda_{max}=252$ nm. EIMS (high resolution) calcd for $C_7H_{11}N_3O_2$ 169.08418, found 169.08513. ^1H NMR (300 MHz, DMSO-d_6) δ: 0.91 (t, $J=7.3$ Hz, 3H, CH_3), 1.56 (m, $J=7.3$ Hz, 2H, CH_2), 2.76 (t, $J=7.3$ Hz, 2H, CH_2), 3.95 (s, 1H, C^5-NH), 6.30 (s, 1H, H-6), 7.89 (s, 1H, N-1), 10.39 (s, 1H, N-3). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 21.4, 40.1, 45.2, 112.1, 123.8, 149.1, 161.1.
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8. Analytical HPLC was carried out on a XTerra RP_{18} reverse phase column (4.6×150 mm, 5 μm) using water–20 mM potassium dihydrogen phosphate pH 2.5 (90:10 v/v) as eluent or water–methanol for HPLC-MS.
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15. Compound **9a**: UV (in 20 mM aqueous KH_2PO_4 pH 2.5): $\lambda_{max\ 1}=219$ nm, $\lambda_{max\ 2}=276$ nm. EIMS (high resolution) calcd for $C_5H_8N_4O$ 140.07091, found 140.06981. ^1H NMR (300 MHz, DMSO-d_6) δ: 2.25 (s, 3H, CH_3), 3.30 (s, 1H, C^5-NH), 4.90 (s, 1H, H-6), 5.23 (s, 2H, NH_2), 6.70 (s, 1H, N-1). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 30.7, 39.8, 119, 153.3, 159.6. Compound **9b**: UV (in 20 mM aqueous KH_2PO_4 pH 2.5): $\lambda_{max\ 1}=205$ nm, $\lambda_{max\ 2}=270$ nm. EIMS (high resolution) calcd for $C_6H_{10}N_4O$ 154.08551, found 154.08546. ^1H NMR (300 MHz, DMSO-d_6) δ: 1.15 (t, $J=7.2$ Hz, 3H, CH_3), 2.81 (q, $J=7.2$ Hz, 2H, CH_2), 6.52 (s, 1H, C^5-NH), 7.89 (s, 2H, NH_2), 10.12 (s, 1H, N-1). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 14.2, 39.2, 117.3, 120.4, 155.2, 161.0. Compound **9c**: UV (in 20 mM aqueous KH_2PO_4 pH 2.5): $\lambda_{max}=276$ nm. EIMS (high resolution) calcd for $C_7H_{12}N_4O$ 168.10239, found 168.10110. ^1H NMR (300 MHz, DMSO-d_6) δ: 0.91 (t, $J=7.2$ Hz, 3H, CH_3), 1.30 (m, $J=7.2$ Hz, 2H, CH_2), 2.80 (t, $J=7.2$ Hz, 2H, CH_2), 4.12 (s, 1H, C^5-NH), 6.40 (s, 1H, H-6), 7.70 (s, 1H, NH_2), 10.22 (s, 1H, N-1). ^{13}C NMR (300 MHz, DMSO-d_6) δ: 21.8, 39.2, 46.2, 117.2, 120.2, 155.0, 161.0.