

## THE PHOTOCHEMISTRY OF PYRIMIDINE IN REALISTIC ASTROPHYSICAL ICES AND THE PRODUCTION OF NUCLEOBASES

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### ABSTRACT

Nucleobases, together with deoxyribose/ribose and phosphoric acid, are the building blocks of DNA and RNA for all known life. The presence of nucleobase-like compounds in carbonaceous chondrites delivered to the Earth raises the question of an extraterrestrial origin for the molecules that triggered life on our planet. Whether these molecules are formed in interstellar/protostellar environments, in small parent bodies in the solar system, or both, is currently unclear. Recent experiments show that the UV irradiation of pyrimidine (C<sub>4</sub>H<sub>4</sub>N<sub>2</sub>) in H<sub>2</sub>O-rich ice mixtures that contain NH<sub>3</sub>, CH<sub>3</sub>OH, or CH<sub>4</sub> leads to the formation of the pyrimidine-based nucleobases uracil, cytosine, and thymine. In this work, we discuss the low-temperature UV irradiation of pyrimidine in realistic astrophysical ice mixtures containing H<sub>2</sub>O, CH<sub>3</sub>OH, and NH<sub>3</sub>, with or without CH<sub>4</sub>, to search for the production of nucleobases and other prebiotic compounds. These experiments show the presence of uracil, urea, glycerol, hexamethylenetetramine, small amino acids, and small carboxylic acids in all samples. Cytosine was only found in one sample produced from ices irradiated with a higher UV dose, while thymine was not found in any sample, even after irradiation with a higher UV dose. Results are discussed to evaluate the role of the photochemistry of pyrimidine in the inventory of organic molecules detected in meteorites and their astrophysical/astrobiological implications.

*Key words:* astrobiology – astrochemistry – ISM: molecules – methods: laboratory: molecular – molecular processes – ultraviolet: ISM

### 1. INTRODUCTION

Nucleobases are the informational subunits of RNA and DNA that carry the genetic code of all living systems on Earth. From a structural point of view, they are nitrogen heterocycles that are divided into two families, according to the carbonaceous backbone on which their molecular structure is based. Pyrimidine (C<sub>4</sub>H<sub>4</sub>N<sub>2</sub>) serves as the backbone for the nucleobases uracil, cytosine, and thymine, while purine (C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>) serves as the backbone for adenine and guanine. Nitrogen heterocycles, including nucleobases, have been detected in a number of carbonaceous chondrites such as Murchison, Murray, Orgueil, and Lonewolf Nunataks 94102 (Folsome et al. 1971; Hayatsu et al. 1975; Stoks & Schwartz 1979; Callahan et al. 2011), and their extraterrestrial origin is supported by isotopic measurements (Martins et al. 2008). To date, no individual *N*-heterocycles have been detected in the gas phase of the interstellar medium (ISM) despite extensive astronomical observation efforts (Simon & Simon 1973; Kuan et al. 2003; Charnley et al. 2005). However, analysis of the emission features in astrophysical environments associated with polycyclic aromatic hydrocarbons (PAHs) suggests that *N*-heterocycles—sometimes referred to as polycyclic aromatic nitrogen heterocycles (PANHs)—may constitute part of the PAH population (Mattioda et al. 2003; Bernstein et al. 2005). As is the case for PAHs, PANHs are expected to be condensed on the surface of cold grains, mixed with ices in dense cloud environments and in colder parts of protostellar disks (Sandford et al. 2004; Bernstein et al. 2005).

Several mechanisms have been proposed for the formation of *N*-heterocycles under astrophysical conditions. For example, they may form via a similar pathway as PAHs in the gas phase, from the polymerization of acetylene (C<sub>2</sub>H<sub>2</sub>) and cyanic acid (HCN) and/or isocyanic acids (HNC; Frenklach & Feigelson 1989; Ricca et al. 2001). Cyclic *N*-bearing molecules such

as fully hydrogenated pyrimidine and 1,3,5-triazine derivatives were also shown to be produced from the UV irradiation of astrophysical ice analogs containing H<sub>2</sub>O, CH<sub>3</sub>OH, NH<sub>3</sub>, CO, and CO<sub>2</sub> at low temperature, although none of these compounds is aromatic (Meierhenrich et al. 2005).

Numerous laboratory studies have shown that ultraviolet (UV) photoprocessing of ice mixtures of astrophysical interest containing H<sub>2</sub>O, CH<sub>3</sub>OH, CO, CO<sub>2</sub>, NH<sub>3</sub>, etc., at low temperatures (<20 K) leads to the formation of complex organic molecules. These photoproducts are embedded in a residue that is stable at 300 K and can be recovered after the sample is warmed (e.g., Bernstein et al. 1995; Muñoz Caro & Schutte 2003). Analysis of these residues reveals that they contain a wide variety of organic compounds including molecules of biological interest such as amino acids, urea, glycerol, and amphiphiles (Dworkin et al. 2001; Bernstein et al. 2002a; Muñoz Caro et al. 2002; Nuevo et al. 2008, 2010). The addition of PAHs to these ices leads to the formation of aromatic molecules that contain a wide range of functional groups on their peripheral carbon atoms (Bernstein et al. 1999, 2002b). Similarly, the addition of pyrimidine to H<sub>2</sub>O-rich binary ice mixtures that contain NH<sub>3</sub>, CH<sub>3</sub>OH, or CH<sub>4</sub> leads to the formation of the nucleobases uracil, cytosine, and thymine (Nuevo et al. 2009, 2012; Materese et al. 2013). In these experiments, thymine was only formed in much smaller quantities, and only when using UV photon doses three times higher than those to form uracil and cytosine. The low level of thymine production may be a result of the larger number of chemical substitutions required for its formation relative to uracil and cytosine (Materese et al. 2013). In addition, it also appears that methyl group addition does not compete well with oxidation (Sandford et al. 2014).

Interestingly, it is believed that the first terrestrial molecules capable of self-replication were based on RNA (Gilbert 1986; Joyce 1989), which does not require any thymine. In a scenario

in which these first RNA-based chemical reactions leading to the emergence of life on the primitive Earth were triggered by organic materials delivered via meteorites and comets (Oró 1961; Chyba & Sagan 1992), thymine may have had a very limited role. This is consistent with the difficulties observed to produce thymine in ice irradiation experiments, and with the fact that it has yet to be detected in meteorites.

Our earlier work on pyrimidine-containing ices were mainly aimed at establishing the chemical pathways by which these molecules were made in simple ices, and we did not carry out experiments designed to simulate the irradiation of pyrimidine in more realistic astrophysical ice analogs. In this work, we have performed a series of experiments of UV irradiation (with two photon dose levels) of pyrimidine in ice mixtures of compositions  $\text{H}_2\text{O}:\text{CH}_3\text{OH}:\text{NH}_3$  and  $\text{H}_2\text{O}:\text{CH}_3\text{OH}:\text{CH}_4:\text{NH}_3$ . The relative proportions between the ice components in these ices were chosen to be consistent with observations of ices in several astrophysical environments (Dartois et al. 1999, 2002; Ehrenfreund & Charnley 2000; Gibb et al. 2004; Bottinelli et al. 2010; Whittet et al. 2011), with the proportion of  $\text{CH}_3\text{OH}$  at the high end of observed values in order to increase the signal for some newly formed compounds in the final samples (see Section 2). The resulting residues were recovered at room temperature and analyzed to search for the presence of pyrimidine-based photoproducts including uracil, cytosine, and thymine, together with other compounds of biological interest. The results are compared with the inventory of molecules found in carbonaceous chondrites and discussed from an astrophysical point of view to determine the role of ice photochemistry in establishing the inventory of materials that were delivered to the early Earth—and by extension, any planet—and may have contributed to the emergence of life.

## 2. EXPERIMENTAL SETUP AND METHODS

### 2.1. Irradiation of Ices

A detailed description of the procedure used for the preparation and irradiation of our samples can be found in Nuevo et al. (2009). Briefly, premixed gas mixtures were injected in a vacuum cryogenic chamber evacuated to a pressure of a few  $10^{-8}$  mbar and deposited onto a piece of aluminum foil (pre-baked to 500°C) attached to a cold finger cooled to 15–20 K by a closed-cycle helium cryocooler. The gas mixtures used were prepared in a glass line evacuated to  $\sim 10^{-6}$  mbar by mixing  $\text{H}_2\text{O}$  vapor,  $\text{CH}_3\text{OH}$  vapor,  $\text{CH}_4$  gas,  $\text{NH}_3$  gas, and pyrimidine vapor in 2.1 L glass bulbs. Purities of these compounds are given elsewhere (Nuevo et al. 2009, 2012; Materese et al. 2013). Ratios between the components of these mixtures were determined by their partial pressure in the bulb ( $\pm 0.05$  mbar). In this work, we prepared mixtures of two different compositions, namely,  $\text{H}_2\text{O}:\text{CH}_3\text{OH}:\text{NH}_3:\text{C}_4\text{H}_4\text{N}_2$  in relative proportions 20:10:2:1 (hereafter M1) and  $\text{H}_2\text{O}:\text{CH}_3\text{OH}:\text{CH}_4:\text{NH}_3:\text{C}_4\text{H}_4\text{N}_2$  in relative proportions 20:10:5:2:1 (hereafter M2). These relative proportions were chosen to mimic the abundances of  $\text{H}_2\text{O}$ ,  $\text{CH}_3\text{OH}$ ,  $\text{CH}_4$ , and  $\text{NH}_3$  in cold interstellar clouds and comets (Dartois et al. 1999, 2002; Ehrenfreund & Charnley 2000; Gibb et al. 2004; Bottinelli et al. 2010; Whittet et al. 2011). It must however be noted that the relative proportion of  $\text{CH}_3\text{OH}$  in the ice mixtures was placed at the higher end of concentrations seen in protostellar/dense clouds environments in order to maximize the signal-to-noise ratio for some of the peaks detected in the GC-MS chromatograms and corresponding to newly formed C-bearing species, in particular pyrimidine derivatives contain-

ing  $\text{CH}_3$  and  $\text{CH}_2\text{OH}$  groups (see Section 2.2). Similarly, the relative proportion of pyrimidine, not detected in astrophysical environments, was selected to be artificially higher than its expected abundance in order to increase the detectability of the resulting pyrimidine-based photoproducts in the same chromatograms (Section 3).

Simultaneous with deposition, the ice films were irradiated with UV photons from an F-shaped microwave-powered  $\text{H}_2$ -discharge UV lamp (Ophos), with a  $\text{H}_2$  pressure of about 0.15 torr (0.2 mbar). The UV spectrum provided by this lamp consists of a strong Ly- $\alpha$  (121.6 nm) emission and a series of  $\text{H}_2$  transition bands in the 155–165 nm range (Chen et al. 2014), with an estimated flux of  $\sim 2 \times 10^{15}$  photons  $\text{cm}^{-2} \text{s}^{-1}$  (Warnek 1962). The light emitted by such a lamp is a good analog to the UV radiation field found in many astrophysical environments including the dense ISM and protostars (d’Hendecourt et al. 1986; Allamandola et al. 1988; Bernstein et al. 1995; Cruz-Diaz et al. 2014). In this work, the photon doses received by the samples were either 0.25 photons molecule $^{-1}$  (hereafter referred to as the lower UV dose) or 0.70 photons molecule $^{-1}$  (hereafter referred to as the higher UV dose).

Assuming a typical photon flux for the diffuse ISM of  $8 \times 10^7$  photons  $\text{cm}^{-2} \text{s}^{-1}$  for photons with energies higher than 6 eV (Mathis et al. 1983), and fluxes expected to be 3–5 orders of magnitude lower in dense interstellar clouds (Prasad & Tarafdar 1983; Shen et al. 2004), our laboratory experiments correspond to a UV irradiation of ices of about  $10^5$  yr in diffuse media, and  $10^8$ – $10^{10}$  yr in dense media, which are reasonable astrophysical scales for our experiments to be compared with. It should be noted, however, that a recent modeling study showed that ice-coated grains in protostellar disks may experience much higher UV photon fluxes, as each grain may be exposed to an average of  $5 \times 10^{12}$  photons over a period of  $10^6$  yr (Ciesla & Sandford 2012).

Control experiments, in which (1) an M2 ice mixture was deposited following the same procedure but not UV irradiated, (2) an aluminum foil with no ices was UV irradiated, and (3) pure pyrimidine was deposited and UV irradiated, were also performed for comparison with residues produced from irradiated ices. These controls showed no evidence for the presence of materials beyond those of pyrimidine in the case of the deposition without UV irradiation.

After deposition and irradiation, samples were warmed under static vacuum to room temperature, at which time they were removed from the vacuum chamber. Al foils were recovered from the cold finger and put in clean, prebaked (500°C) vials containing 500  $\mu\text{L}$  of  $\text{H}_2\text{O}$  in which the resulting residues were dissolved. Residues resulting from the irradiation of the ice mixtures of M1 and M2 compositions with the lower photon dose will hereafter be referred to as M1<sub>L</sub> and M2<sub>L</sub>, respectively, while those from the irradiation with the higher photon dose will be referred to as M1<sub>H</sub> and M2<sub>H</sub>, respectively.

### 2.2. Analysis of Residues

The chromatographic analyses of the residues obtained in this study follow the same protocol used for our earlier study of UV irradiation of pyrimidine in  $\text{H}_2\text{O}$ -rich ices (Nuevo et al. 2009, 2012; Materese et al. 2013). Briefly, 100  $\mu\text{L}$  of each sample was dried under vacuum for 2 hr and derivatized with MTBSTFA. Then 1  $\mu\text{L}$  of the derivatized fraction of each residue was injected into a gas chromatograph coupled to a mass spectrometer (GC-MS) equipped with the same Restek Rxi-5ms column and applying the same temperature gradient

as for our previous studies (Nuevo et al. 2009, 2012; Materese et al. 2013). This column and protocol are optimized to search for the presence of small aromatic photoproducts containing OH and NH<sub>2</sub> groups, including the nucleobases uracil, cytosine, and thymine. Most of the results presented here were provided by Rxi-5ms data.

Underivatized fractions of the samples were dried under vacuum and dissolved in a 1:1 mixture of methanol and acetonitrile, from which 1  $\mu$ L of each was directly injected into a GC-MS Restek Rtx-200MS column to search for other photoproducts that cannot be derivatized with MTBSTFA, including methylpyrimidines. This GC-MS method used splitless injection, with an injector temperature of 250°C, and a carrier gas (He) flow rate of 1.0 mL min<sup>-1</sup>. The temperature was initially held at 40°C for 4 minutes, then ramped at 6°C min<sup>-1</sup> to 250°C, and finally held at 250°C for 10 minutes.

Photoproducts in the sample chromatograms were identified via both their retention times in the columns and their mass spectra by comparison with commercial standards prepared and injected under the same conditions as the samples (Nuevo et al. 2009, 2012; Materese et al. 2013). Masses in the mass spectrometer were recorded from 19 to 500 Da, and data analysis was performed with the Xcalibur<sup>TM</sup> software (Thermo Finnigan).

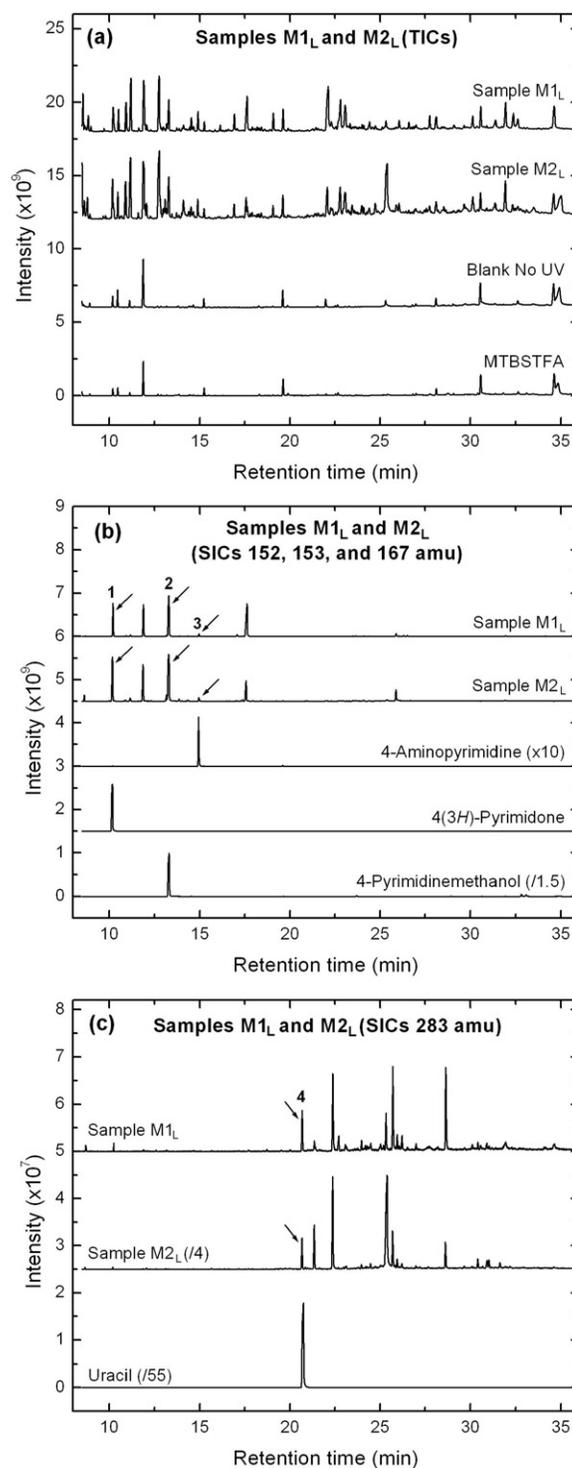
### 3. RESULTS AND DISCUSSION

#### 3.1. Identification of Nucleobases

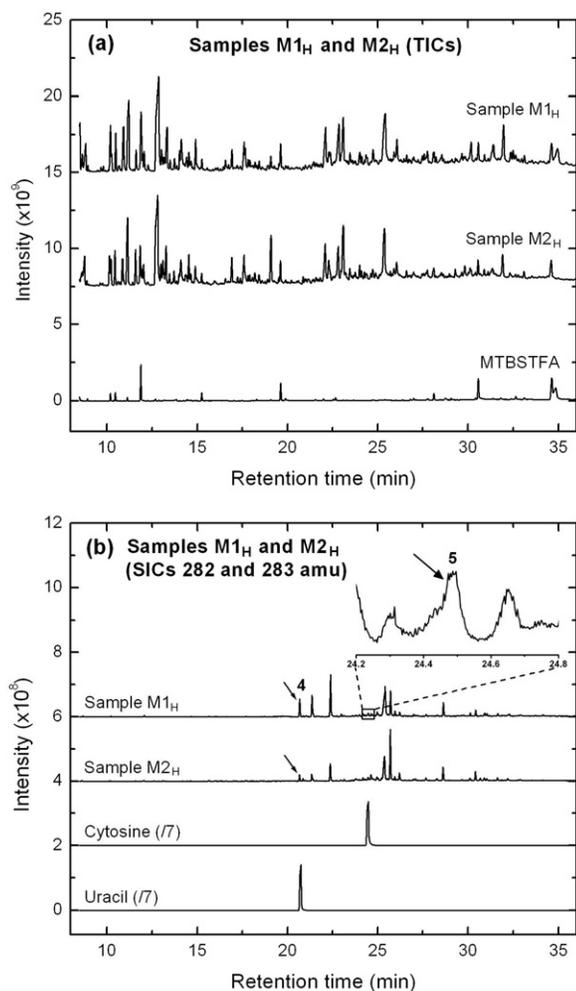
The UV irradiation of mixtures M1 and M2 with the lower photon dose leads to the formation of a wide variety of photoproducts, as shown by the large number of peaks in the GC-MS chromatograms (Rxi-5ms column) of their resulting residues, M1<sub>L</sub> and M2<sub>L</sub> (Figure 1(a), top two traces). The chromatogram of the control sample in which an M2 ice was not irradiated (blank no UV, bottom middle trace) did not show any photoproducts. Likewise, the chromatogram of the agent used for derivatizing the samples and the standards (MTBSTFA) did not show any peaks other than the characteristic peaks due to its own fragmentation in the mass spectrometer (bottom trace).

Most of the photoproducts identified in the residues M1<sub>L</sub> and M2<sub>L</sub> are the same as those formed after UV irradiation of pyrimidine in pure H<sub>2</sub>O ice and/or in H<sub>2</sub>O + NH<sub>3</sub>, H<sub>2</sub>O + CH<sub>3</sub>OH, and H<sub>2</sub>O + CH<sub>4</sub> ice mixtures (Nuevo et al. 2009, 2012; Materese et al. 2013). These photoproducts are mainly variants of pyrimidine derivatives formed from the addition of peripheral groups such as OH, NH<sub>2</sub>, CH<sub>3</sub>, CH<sub>2</sub>OH, or some combination of these. Identified photoproducts include 4(3*H*)-pyrimidone (a singly oxidized pyrimidine and the main precursor of uracil), 4-aminopyrimidine (a singly aminated pyrimidine and a precursor of cytosine), and 4-pyrimidinemethanol (pyrimidine with a CH<sub>2</sub>OH group added to position 4; Figure 1(b)). They also include three bipyrimidine isomers, variants of hydrogenated pyrimidine such as 1,4,5,6-tetrahydropyrimidine, and the nucleobase uracil (Figure 1(c)). The nucleobases cytosine and thymine were not detected in any of the residues M1<sub>L</sub> or M2<sub>L</sub>.

Interestingly, the relative abundance of 4(3*H*)-pyrimidone (the most abundant oxidized photoproduct) to 4-aminopyrimidine (most abundant aminated photoproduct) in the residues M1<sub>L</sub> and M2<sub>L</sub> almost exactly matched the relative proportion of available OH (from H<sub>2</sub>O and CH<sub>3</sub>OH) and NH<sub>2</sub> groups (from NH<sub>3</sub>) in the starting ices, i.e., about 15:1. This result suggests that •OH and •NH<sub>2</sub> radicals are produced from their parent molecules in the ices with very similar



**Figure 1.** (a) From top to bottom, GC-MS total-ion chromatograms (TICs) of a residue M1<sub>L</sub> and a residue M2<sub>L</sub> produced from the UV irradiation of their respective starting ice mixtures M1 (H<sub>2</sub>O:CH<sub>3</sub>OH:NH<sub>3</sub>:C<sub>4</sub>H<sub>4</sub>N<sub>2</sub> = 20:10:2:1) and M2 (H<sub>2</sub>O:CH<sub>3</sub>OH:CH<sub>4</sub>:NH<sub>3</sub>:C<sub>4</sub>H<sub>4</sub>N<sub>2</sub> = 20:10:5:2:1) with the lower photon dose, of a control experiment (blank) in which an M2 ice was not UV irradiated, and of the derivatizing agent (MTBSTFA) for comparison. (b) From top to bottom, single-ion chromatograms (SICs) of the same residues for *m/z* = 152 amu (pyrimidine + one NH<sub>2</sub> group), 153 amu (pyrimidine + one OH group), and 167 amu (pyrimidine + one CH<sub>2</sub>OH group), and of the 4-aminopyrimidine (peak 3, 152 amu), 4(3*H*)-pyrimidone (peak 1, 153 amu), and 4-pyrimidinemethanol (peak 2, 167 amu) standards. (c) From top to bottom, SICs of the same residues for *m/z* = 283 amu (pyrimidine + two OH groups) and of the uracil standard (peak 4, 283 amu). Detections of the photoproducts in the residues are marked with arrows.



**Figure 2.** (a) From top to bottom, GC-MS TICs of a residue M1<sub>H</sub> and a residue M2<sub>H</sub> produced from the UV irradiation of their respective starting ice mixtures M1 (H<sub>2</sub>O:CH<sub>3</sub>OH:NH<sub>3</sub>:C<sub>4</sub>H<sub>4</sub>N<sub>2</sub> = 20:10:2:1) and M2 (H<sub>2</sub>O:CH<sub>3</sub>OH:CH<sub>4</sub>:NH<sub>3</sub>:C<sub>4</sub>H<sub>4</sub>N<sub>2</sub> = 20:10:5:2:1) with the higher photon dose, and of MTBSTFA for comparison. (b) From top to bottom, SICs of the same residues for  $m/z = 282$  amu (pyrimidine + one NH<sub>2</sub> group + OH group) and 283 amu (pyrimidine + two OH groups), and of the cytosine (282 amu) and uracil (283 amu) standards. Detections of uracil (peak 4) and cytosine (enlargement; peak 5) are marked with arrows.

efficiencies, as expected from the comparable dissociation energies of HO–H (5.1 eV) and NH<sub>2</sub>–H (4.6 eV) (Mordaunt et al. 1996; Woon 2002), and that the efficiencies of OH and NH<sub>2</sub> nucleophilic additions to pyrimidine are comparable.

Irradiation of the starting ices with a three-fold increase in UV dose (residues M1<sub>H</sub> and M2<sub>H</sub>; Figure 2) resulted in chromatograms that display a larger total number of peaks, although the overall retention pattern is similar (Figure 2(a), top and middle traces). Despite the fact that the identities of these additional peaks could not be formally determined, their mass spectra indicate that they are mostly different isomers of the substituted pyrimidines already observed in the residues produced from the irradiation of ices with the lower dose. The higher UV dose also affected the relative abundances of certain species in the resulting residues compared with those produced from the lower UV dose. For example, the relative abundance of uracil to its precursor 4(3*H*)-pyrimidone was found to increase by an order of magnitude when ices were irradiated with the higher photon dose, suggesting that the use of a higher dose results in the production of more substituted photoproducts.

Moreover, the relative abundance of 4-aminopyrimidine to 4(3*H*)-pyrimidone was found to be 2.5–3 times higher for the higher UV dose. This may have resulted in the formation of larger amounts of cytosine, allowing its unambiguous detection in one of the M1<sub>H</sub> residues, produced with the higher UV dose (Figure 2(b), top trace and enlargement). However, no cytosine was detected in any of the M2<sub>H</sub> residues. In all cases, no thymine was detected in any of the residues, regardless of the composition of the starting ices and of the UV photon dose employed to irradiate the ices, although Materese et al. (2013) recently showed that thymine can be formed when pyrimidine is mixed with an H<sub>2</sub>O + CH<sub>4</sub> ice and UV irradiated with the higher photon dose.

The photoproducts identified in the residues and formed from the successive additions of OH, NH<sub>2</sub>, and CH<sub>3</sub> groups to pyrimidine are summarized in Figure 3. The molecular structures of all other compounds mentioned in the text but not shown in Figure 3 can be found elsewhere (Nuevo et al. 2009, 2012; Materese et al. 2013).

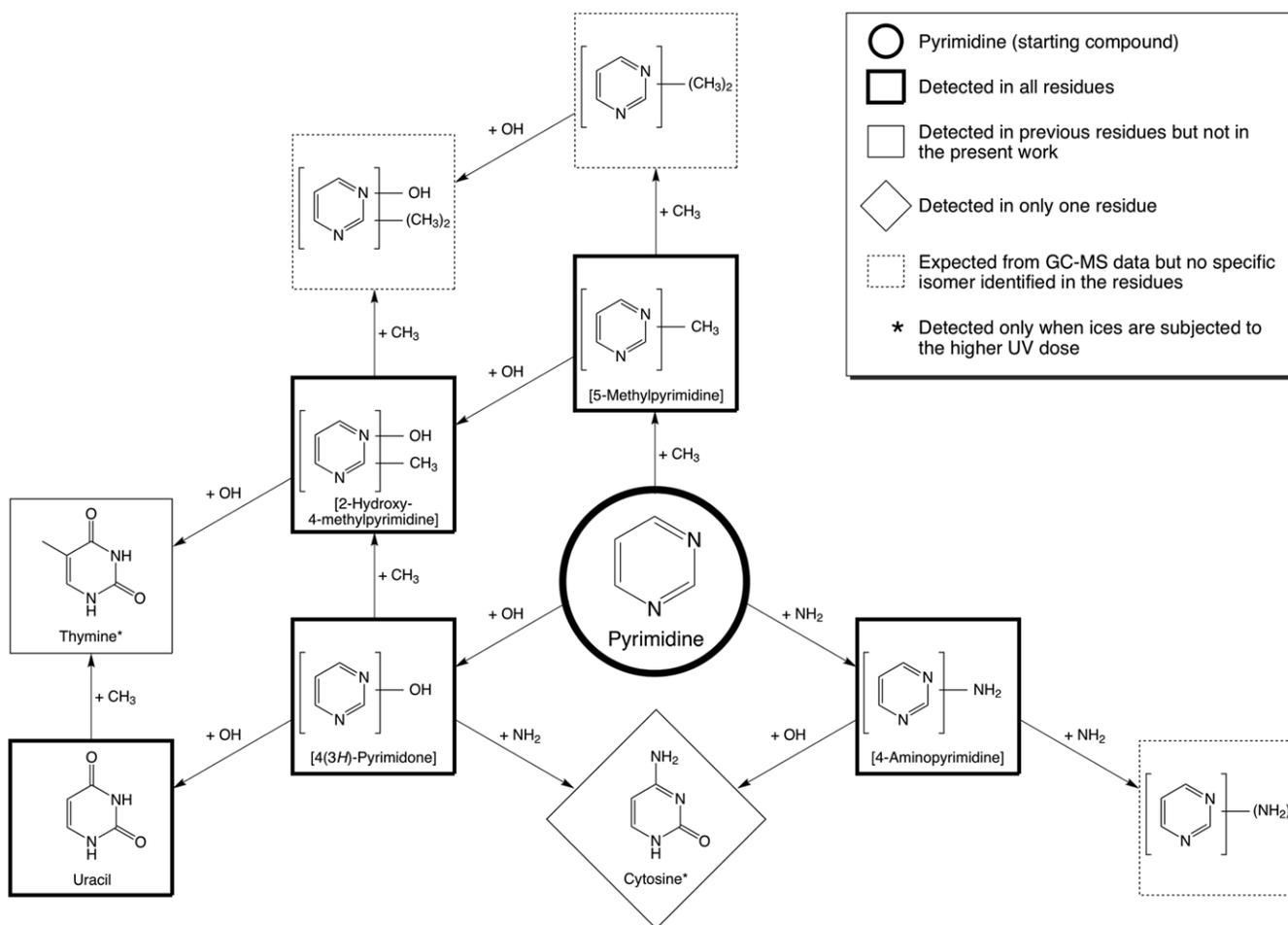
### 3.2. Mechanisms of Formation of Nucleobases

Even at low temperatures such as those in which ice mixtures are irradiated in our experiments, the formation of pyrimidine derivatives via additions of OH, NH<sub>2</sub>, and CH<sub>3</sub> groups—as well as other groups including CH<sub>2</sub>OH—to pyrimidine may not result from the straightforward, successive addition of groups to pyrimidine. Rather, the formation of the nucleobases uracil, cytosine, thymine, and their precursors may be seen as the final result of a succession of additions and dissociations. Under this stochastic process, the number of absorbed UV photons that result in the formation of singly substituted pyrimidine derivatives (e.g., 4(3*H*)-pyrimidone and 4-aminopyrimidine) is probably larger than one, and the number of absorbed photons that result in the formation of doubly (e.g., uracil and cytosine) or triply (e.g., thymine) substituted pyrimidines likely increases in a nonlinear manner.

Following this reasoning, and assuming that group addition is comparable for different groups, uracil and cytosine (two added groups) would be expected to form with comparable abundances, while thymine (three added groups) would be expected to form in significantly smaller amounts. In these experiments, uracil was the only nucleobase detected in the residues of photoprocessed ices, regardless of the starting mixture or radiation dose. Its observed molecular yield was comparable to what was observed in previous experiments involving the irradiation of pyrimidine in pure H<sub>2</sub>O ice and binary H<sub>2</sub>O-rich ice mixtures (Nuevo et al. 2009, 2012; Materese et al. 2013).

Cytosine was unambiguously detected in only one M1<sub>H</sub> residue produced from the irradiation of an M1 ice mixture (no CH<sub>4</sub>) with the higher UV photon dose (Figure 2(b)). This result may be due to either or both of the following reasons: (1) the low abundance of NH<sub>3</sub> compared with H<sub>2</sub>O in the starting ice mixtures—about 1:10—may result in the formation of cytosine with abundances too low to be detected, or (2) cytosine is formed with comparable abundances to uracil, before it is eventually converted into uracil via hydrolysis. Interestingly, the conversion of cytosine into uracil via hydrolysis was previously studied to better understand the non-detection of cytosine in carbonaceous chondrites (Shapiro 1999; Nelson et al. 2001).

The absence of thymine in the residues produced from the UV irradiation of realistic astrophysical ice mixtures suggests



**Figure 3.** Diagram summarizing the formation of pyrimidine derivatives from the successive additions of OH, NH<sub>2</sub>, and CH<sub>3</sub> groups to pyrimidine (in the center). Photoproducts detected in the residues are in bolded boxes, and include uracil and cytosine, as well as their precursors 4(3*H*)-pyrimidone and 4-aminopyrimidine. Cytosine was unambiguously detected in only one residue M1<sub>H</sub>, while thymine was not detected in any residue. Names of photoproducts given between brackets correspond to the most abundant isomers of each family of compounds identified. Dashed boxes indicate that GC-MS data of the residues are consistent with photoproducts of these masses, but no specific isomer could be identified.

that the addition of the three specific groups necessary to form thymine is in competition with a large number of other possible reactions in the ices, leading to a very low production yield of thymine. One factor that may account for this result is that CH<sub>3</sub>OH, the second most abundant ice component both in our starting mixtures and in many astrophysical environments (Dartois et al. 1999, 2002; Ehrenfreund & Charnley 2000; Gibb et al. 2004; Bottinelli et al. 2010; Whittet et al. 2011), is not a good source of •CH<sub>3</sub> radicals. Experiments carried out by Öberg et al. (2009) showed that when subjected to UV radiation, CH<sub>3</sub>OH dissociates into •CH<sub>2</sub>OH, •OCH<sub>3</sub>, and •CH<sub>3</sub>/•OH radicals with relative proportions 5:1:1. This is in agreement with the photoproducts identified in our residues, as very few methylated pyrimidines were found, while 4-pyrimidinemethanol was one of the most abundant photoproducts formed (Figure 1(b)). Even in residues produced from the irradiation of an M2 ice mixture, which contained CH<sub>4</sub>, the production yields for methylated pyrimidines were very low and comparable to those for residues produced from an M1 ice mixture (with no CH<sub>4</sub>). This suggests that the methylation of pyrimidine competes poorly with oxidation or amination in H<sub>2</sub>O-rich ice mixtures, which is in agreement with recent experiments involving the irradiation of pyrimidine in H<sub>2</sub>O + CH<sub>3</sub>OH and H<sub>2</sub>O + CH<sub>4</sub> ices (Materese et al. 2013; Sandford et al. 2014).

### 3.3. Identification of Other Compounds

The UV irradiation of both M1 and M2 ice mixtures also led to the formation of a variety of nonpyrimidine-based photoproducts which include urea, glycerol, hydantoin, hexamethylenetetramine (HMT), small biological (glycine, alanine) and nonbiological ( $\beta$ -alanine, *N*-formylglycine) amino acids, as well as small carboxylic acids (glycolic acid, oxalic acid, lactic acid, and 3-hydroxypropanoic acid). Among these compounds, HMT is the only one that was not identified by comparison with a standard, but was identified using the Xcalibur<sup>TM</sup> analysis software and its built-in NIST database.

Most of these photoproducts are known to form via UV irradiation of ice mixtures containing CH<sub>3</sub>OH and NH<sub>3</sub> (Bernstein et al. 1995; Muñoz Caro & Schutte 2003; Nuevo et al. 2008, 2012; de Marcellus et al. 2011), which are both present in the starting M1 and M2 ice mixtures, and likely do not require pyrimidine to form. For example, glycerol is most probably formed from the combination of three CH<sub>3</sub>OH molecules. Nevertheless, previous experiments show that urea, glycine, alanine, and the same carboxylic acids as those detected in the present study are formed when pyrimidine is irradiated in H<sub>2</sub>O + NH<sub>3</sub> ice mixtures (Nuevo et al. 2012). This indicates that the pyrimidine ring can be ruptured and become a source of carbon and nitrogen for the production of other organic molecules.

Of particular interest is the formation of HMT in all our residues, as it is known to be the precursor of a large variety of organic molecules (Bernstein et al. 1995). Its formation mechanism requires the presence of  $[(\text{XCOO}^-)(\text{NH}_4^+)]$  salts at room temperature (Muñoz Caro & Schutte 2003), where  $\text{XCOO}^-$  denotes the anionic (carboxylate) form of a carboxylic acid. Any or all of the carboxylic acids identified in our residues could play the role of the carboxylate  $\text{XCOO}^-$ , while the ammonium cation  $\text{NH}_4^+$  is known to be present in residues resulting from the photoprocessing of  $\text{NH}_3$ -containing ices (Bernstein et al. 1995; Muñoz Caro & Schutte 2003; Nuevo et al. 2006). This suggests that HMT may be formed from several precursors.

### 3.4. Astrophysical Implications

Assuming that the starting ices of compositions M1 and M2 are realistic analogs of the ices observed in the ISM and comets, the results of our experiments are interesting from an astrophysical point of view. Since interstellar and cometary ices are dominated by  $\text{H}_2\text{O}$ , it is reasonable to assume that a large number of  $\bullet\text{OH}$  radicals will be created in the ices upon UV photoirradiation. Even at the very low temperatures of cold molecular clouds ( $<50$  K), radicals are reactive species that can engage in nearly barrierless reactions with their close neighbors in the ice matrix to form increasingly complex compounds.

As  $\text{CH}_3\text{OH}$  is usually the second most abundant component in astrophysical ices (Dartois et al. 1999, 2002; Ehrenfreund & Charnley 2000; Gibb et al. 2004; Bottinelli et al. 2010; Whittet et al. 2011) and its presence also contributes to the production of  $\bullet\text{OH}$  radicals, oxidation is expected to be one of the dominant chemical processes taking place in the ices. Similarly, although not as abundant when compared with  $\text{H}_2\text{O}$  or  $\text{CH}_3\text{OH}$ ,  $\text{NH}_3$  can be readily photolyzed to form  $\bullet\text{NH}_2$  radicals that can efficiently react with nearby species. The high efficiency of the UV-photoinduced incorporation of nitrogen into complex organic molecules was previously observed in residues produced from the irradiation of ices containing  $\text{H}_2\text{O}$ ,  $\text{CH}_3\text{OH}$ ,  $\text{CO}$ , and  $\text{NH}_3$  (Nuevo et al. 2011). Indeed, the elemental ratios N/C in those residues were measured to be 7–16 times higher than those in the starting ices. Consequently, if pyrimidine is present in astrophysical  $\text{H}_2\text{O}$ -,  $\text{CH}_3\text{OH}$ -, and  $\text{NH}_3$ -containing ices, uracil and cytosine, as well as their precursors 4(3*H*)-pyrimidone and 4-aminopyrimidine would be expected to be formed in nonnegligible quantities. This result is verified in the present experiments, as all these photoproducts were detected in all the residues, with the exception of cytosine, which was only unambiguously detected in one residue M1<sub>H</sub> (higher UV dose).

While OH and  $\text{NH}_2$  group additions to pyrimidine are efficient processes, this is not the case for the addition of  $\text{CH}_3$  groups in an  $\text{H}_2\text{O}$ -rich ice matrix. Instead, based on our experimental results, methylation of pyrimidine seems to compete unfavorably with its oxidation, so that the formation of  $\text{CH}_3$ -containing pyrimidine derivatives, including the nucleobase thymine, is partly inhibited (Materese et al. 2013; Sandford et al. 2014). Similarly, since  $\text{NH}_2$  group addition appears to be about as efficient as OH group addition, we anticipate methylation to be further inhibited by the presence of  $\text{NH}_3$  in the ice. In astrophysical ices, methylation is also expected to be limited because the abundance of  $\text{CH}_4$ —the best source of  $\text{CH}_3$  groups—is estimated to be an order of magnitude lower than that of  $\text{CH}_3\text{OH}$  (Dartois et al. 2002; Gibb et al. 2004). Therefore, in an ice mixture simulating the composition of ices in astrophysical en-

vironments, such as the M1 and M2 ices in the present study, the production yields of OH- and  $\text{NH}_2$ -containing pyrimidine derivatives are expected to be significantly higher than those of  $\text{CH}_3$ -containing pyrimidine derivatives.

It is interesting to compare these results with the detection of these molecules in carbonaceous chondrites such as the Murchison meteorite. In these meteorites, oxidized pyrimidines such as 4(3*H*)-pyrimidone and uracil, and  $\text{CH}_2\text{OH}$ -containing pyrimidines such as 4-pyrimidinemethanol have been detected (Folsome et al. 1971). More generally, uracil is the only pyrimidine-based nucleobase that has thus far been reported in meteorites (Stoks & Schwartz 1979; Callahan et al. 2011). In that work, cytosine and thymine were not found, and derived upper limits on the concentration of thymine in Allende, Orgueil, Murchison, and Murray indicated that it would be at least one order of magnitude lower than the concentration of uracil.

While the nondetection of cytosine in meteorites may be explained by its conversion into uracil via hydrolysis during the extraction protocol of meteoritic samples (Shapiro 1999; Nelson et al. 2001), the nondetection of thymine in the same meteorites cannot be explained by a similar hydrolysis conversion. The work presented here may provide clues to explain the nondetection of thymine in meteorites, if we assume that photoinduced processes on astrophysical icy grains play an important role in the production of the complex organic molecules—including *N*-heterocycles and nucleobases—that are incorporated into meteorites during the formation of the Solar System.

Qualitatively, the photoproducts identified in our residues are a good match with the inventory of pyrimidine-based compounds found in meteorites. This includes the detection of uracil and its precursor 4(3*H*)-pyrimidone in all the residues, lower amounts of cytosine, and the nondetection of thymine. In the hypothesis that the origin of life on Earth relied on the extraterrestrial delivery of organic materials abiotically produced in astrophysical environments (Oró 1961; Chyba & Sagan 1992), our results suggest that the nucleobases and many other organic compounds of prebiotic interest may have had contributions from ice photochemistry in the ISM, protoplanetary disks, and/or comets.

It must be noted that these results are mostly applicable to the photochemistry taking place in the dense ISM, in which the number of UV photons received per molecule is expected to be of the same order of magnitude as in our experiments (Prasad & Tarafdar 1983; Shen et al. 2004). However, in the protosolar nebula, it is likely that icy grains received UV radiation doses that are 2–3 orders of magnitude higher than in the ISM (Ciesla & Sandford 2012), so that the production yields of cytosine and thymine may be substantially increased. Nevertheless, even in such a high radiation environment, our experiments suggest that the production of thymine would be expected to be lower than that of uracil and cytosine, because its formation requires one additional step, and because methyl addition reactions will still be inefficient when competing with oxidation and amination.

Interestingly, in all terrestrial life, uracil is only found in RNA, while thymine replaces uracil in DNA. One commonly proposed hypothesis of the origin of life suggests that the first biological reactions required several variants of RNA-based molecules, leading to the emergence of an “RNA world” (Gilbert 1986; Joyce 1989), i.e., in a world in which thymine was not a required part of the molecular inventory. If the detection of pyrimidine-based compounds in meteorites is representative of the inventory of extraterrestrial organics delivered to the primitive Earth, the

high abundance of uracil, lower abundance of cytosine, and absence of thymine may have played a role in the emergence of an “RNA world,” with DNA only appearing at a later stage in the evolving chemical complexity associated with biology.

## 5. CONCLUSIONS

The UV irradiation of pyrimidine in realistic astrophysical ice mixtures of compositions  $\text{H}_2\text{O}:\text{CH}_3\text{OH}:\text{NH}_3$  (20:10:2) and  $\text{H}_2\text{O}:\text{CH}_3\text{OH}:\text{CH}_4:\text{NH}_3$  (20:10:5:2) leads to the formation of a wide variety of compounds in the residues recovered at room temperature after irradiation. These compounds include 4(3*H*)-pyrimidone (a precursor of uracil), 4-aminopyrimidine (a precursor of cytosine), 4-pyrimidinemethanol, and the nucleobase uracil. Cytosine was unambiguously detected in one residue produced from the irradiation of pyrimidine in an  $\text{H}_2\text{O}:\text{CH}_3\text{OH}:\text{NH}_3$  (20:10:2) ice with a photon dose three times higher, while thymine was not detected in any of the residues. The mechanisms of formation of these photoproducts combined with the relative abundances of the ice components in astrophysical environments may explain the differences observed in the relative production yields of uracil, cytosine, and thymine. In particular, the efficiency of oxidation of pyrimidine over its methylation may account for the lack of  $\text{CH}_3$ -containing photoproducts, including thymine, found in the residues.

Interestingly, the photoproducts detected in our residues qualitatively match the inventory of pyrimidine-based compounds found in carbonaceous chondrites. Assuming that photoprocessing of ices in astrophysical environments plays an important role in establishing the organic inventory of protostellar nebulae, including the organic material that is incorporated into the meteorites’ parent bodies, uracil is expected to be significantly more abundant than thymine. This overabundance of uracil over thymine in the organic inventory that seeded the primitive Earth may have favored the emergence of a prebiotic “RNA world” in which biological reactions were dominated by RNA-based compounds, while DNA-based biochemistry may have appeared later in the history of biology.

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