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Infrared Spectral Signatures of Nucleobases in Interstellar Ices II: Pyrimidines

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Abstract

The identification of pyrimidine nucleobases in interstellar environments such as dense clouds and pre-stellar cores represents a much sought-after result in astrochemical and astrobiological research. Indeed, such an identification would allow for insights into the interstellar formation of these complex organic molecules and, possibly, a better understanding of their putative transfer to the early Earth where they may have contributed to the emergence of life. In this present work, we report the results of an extensive mid-infrared spectroscopic study of the solid pyrimidine nucleobases cytosine, thymine, and uracil under conditions representative of the dense interstellar medium to potentially facilitate their identification in such environments. Spectra of the neat crystalline nucleobases, as well as a mixture of all three, were acquired at temperatures of 300 and 20 K. Furthermore, the spectra of these nucleobases in the presence of interstellar ice analogues containing H_2O:NH_3:CH_4:CO:CH_3OH in a 10:1:1:1:1 ratio were also acquired to determine whether any nucleobase absorption features were evident in the presence of ubiquitous volatile interstellar molecular ices, and thus could be used as possible spectroscopic fingerprints for the identification of interstellar pyrimidines. Our present work has identified three mid-infrared absorption bands at 1512, 1237, and 758 cm^{-1} (6.61, 8.08, and 13.19 μm) that could be useful in this regard. The potential utility of these absorption features in observational surveys of the dense interstellar medium, together with the limitations of our study and recommendations for future work, are discussed in detail.

Keywords: astrochemistry; astrobiology; pyrimidines; nucleobases; interstellar medium; infrared spectroscopy

1. Introduction

The pyrimidines are members of a chemical class of heterocyclic organic molecules characterised by a ring structure composed of four carbon atoms and two nitrogen atoms. Three members of this family of molecules, cytosine, thymine, and uracil (Figure 1), play central roles in the life-sustaining chemistry found on Earth, as they are core components of the nucleic acids DNA and RNA. It is therefore perhaps unsurprising that these pyrimidine nucleobases are ubiquitous in terrestrial environments (Rosemeyer, 2004). Cytosine, thymine, and uracil (along with several other nucleobases) have also been identified in fragments of a number of meteorites, including the Murchison, Murray, and Tagish Lake meteorites (Callahan et al., 2011; Burton et al., 2012; Oba et al., 2022), thereby indicating that the formation of these biomolecules may also take place in extraterrestrial environments.

Indeed, previous laboratory work has extensively documented that the processing of organic precursors such as pyrimidine, urea, or formamide under conditions relevant to extraterrestrial environments readily yields pyrimidine nucleobases (Senanayake and Idriss, 2006; Saladino et al., 2005; 2012; 2015; 2016; Nuevo et al., 2009; 2012; 2014a; 2014b; Bera et al., 2010; Materese et al., 2013). The example of formamide is particularly interesting, and the irradiation of this molecule in the presence of meteoritic material using 170 MeV protons at 243 K was shown to yield cytosine, thymine, and uracil (Saladino et al., 2015). A similar result was recorded after the heating of formamide in the presence of olivine minerals representative of interplanetary dust material (Saladino et al., 2005). Moreover, formamide has been shown to augment the formation of nucleotides in experiments concerned with the 170 MeV proton irradiation of nucleosides mixed with meteoritic and phosphorus-rich mineral material at 298 K (Bizzarri et al., 2019). Theoretical studies have also demonstrated energetically favourable pathways towards the formation of nucleobases *via* gas-phase reactions involving formamide, such as that with vinyl cyanide (Jeilani et al., 2013; Lu et al., 2021). Given that formamide has been detected in the interstellar medium (Rubin et al., 1971; Lazareff et al., 1978; Thiel et al., 2017), a region of space characterised by intense radiation and thermal gradients, it is reasonable to assume that pyrimidine nucleobases could form in the icy mantles adsorbed on interstellar dust grains in cold (i.e., 10-20 K) clouds, particularly since atomistic simulations have demonstrated that such ices can offer favourable thermodynamic conditions for the formation of complex organic molecules (Cassone et al., 2021).

This assumption is strengthened by the laboratory work of Oba et al. (2019), who demonstrated that the irradiation of interstellar ice analogues composed of H_2O:NH_3:CO:CH_3OH (5:2:2:2) using Lyman-α photons at 10 K resulted in the production of several nucleobases, including cytosine, thymine, and uracil. Such results complement the findings of other research groups, such as those of Ruf et al. (2019), who also detected the formation of cytosine after the irradiation of a H_2O:NH_3:CH_3OH (2:1:1) interstellar ice analogue at 77 K using Lyman-α photons. It should be noted that both the experimental works of Oba et al. (2019) and Ruf et al. (2019) made use of interstellar ice analogues composed of the most abundant volatile species in space (Boogert et al., 2015; Öberg 2016; McClure et al., 2023), thus further highlighting the proposed favourability of biomolecule synthesis (including pyrimidine nucleobases) under interstellar conditions. Indeed, such results have served as a basis for other groups to assess the radiolytic stability of pyrimidine nucleobases in interstellar ice analogues, with previous experimental work showing that the stability of thymine and uracil is dependent on the composition of the interstellar ice analogue and the temperature at which the irradiation takes place (Materese et al., 2020; Gerakines et al., 2022).

However, despite the overwhelming experimental and theoretical evidence for the abiotic radiation-induced synthesis and chemical behaviour of pyrimidines in interstellar ice analogues, spectroscopic surveys of interstellar targets have yet to positively identify the absorption features of these molecules in observed ices. The lack of a positive detection of interstellar pyrimidines may be due to a number of factors, many of which were described in our previous works (Rosa et al., 2023; 2024a). In particular, the mid-infrared absorption spectra of interstellar targets are so densely populated by absorption bands attributable to the most common volatile species in the icy cosmos (i.e., H_2O, NH_3, CH_4, CO, and CH_3OH; Boogert et al., 2015; Öberg 2016; McClure et al., 2023), that it is very difficult to identify absorption features attributable to more complex but

less abundant molecules (such as pyrimidine nucleobases). However, there do exist regions of these spectra over which these volatile molecular ices do not present any absorption features, and thus the identification of weak absorption features attributable to interstellar complex organic molecules is more promising across these regions (Rosa et al., 2023; 2024a).

Therefore, in this work, we describe the results of an extensive mid-infrared spectroscopic study of the pyrimidine nucleobases cytosine, thymine, and uracil under conditions representative of the dense interstellar medium in which experimental data is provided to determine not only if pyrimidines can be distinguished from other molecular species in observational data acquired across the mid-infrared spectral range, but also to identify specific spectral signatures of these molecules that may be of use to observational astronomers. As part of this study, we have recorded the spectra of these pyrimidines both in their neat form, as well as in the presence of an interstellar ice analogue composed of H_2O:NH_3:CH_4:CO:CH_3OH (10:1:1:1:1) with the aim of identifying any absorption features that are not obscured by those of the more abundant volatile molecular ices and which may therefore be detected by high-resolution and high-sensitivity observatories, such as the *James Webb Space Telescope* (Kalirai 2018). This work also builds upon our previously published research in which three mid-infrared absorption bands at 1255, 940, and 878 cm^{-1} (7.97, 10.64, and 11.39 μm) were identified in the spectra of the purine nucleobases adenine and guanine which were evident even in the presence of abundant quantities of volatile interstellar ice components (Rosa et al., 2023) and thus could perhaps be used as potential spectroscopic signatures of interstellar purines.

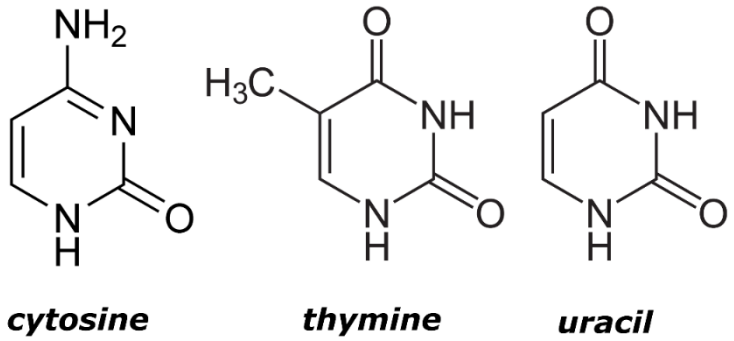

Figure 1. Chemical structures of the pyrimidine nucleobases cytosine, thymine, and uracil.

2. Materials and Methods

The experimental work performed in this study followed a very similar methodology to that in our previous analogous study on purine nucleobases (Rosa et al., 2023). Spectra of pyrimidine nucleobases in the presence of interstellar ice analogues were acquired over the 4000-650 cm^{-1} (15.38-2.50 μm) wavenumber range at a nominal resolution of 1 cm^{-1} using the Ice Chamber for Astrophysics-Astrochemistry (ICA) set-up (Herczku et al., 2021; Mifsud et al., 2021). Briefly, this set-up is an ultrahigh-vacuum chamber operating at a base pressure of 10^{-9} mbar, within the centre of which is a gold-coated oxygen-free high-conductivity copper sample holder which is able to host up to four mid-infrared-transparent ZnSe deposition substrates. The cold finger of a closed-cycle helium cryostat is held in thermal contact with the sample holder and allows it to be cooled to 20 K.

Powdered cytosine and uracil (purity >99% for both) were obtained from Sigma-Aldrich, and powdered thymine (purity >97%) was purchased from Alfa Aesar. These powdered nucleobases were used to prepare diluted solutions. Samples of neat cytosine, neat uracil, and a 1:1:1 three-component mixture of cytosine, thymine, and uracil were diluted in a solution of ethanol in water (60.8% *v/v*), while the sample of neat thymine was dissolved in absolute ethanol. The final sample concentration for each prepared solution was about 1.5 mg mL^{-1}, and approximately 0.4 μL of this solution was dropped onto a ZnSe substrate using a clean pipette. The deposition substrates were then heated to 100 °C to cause the complete evaporation of the solvent, and thus leave behind a 'grainy' solid film of crystalline nucleobases on their surfaces.

The four ZnSe substrates with pyrimidine nucleobases (i.e., neat cytosine, neat thymine, neat uracil, and the three-component mixture) were inserted into the sample holder of the ICA and the chamber was pumped down to base pressure overnight. Mid-infrared absorption spectra of the pyrimidine samples were then acquired at 300 K, prior to cooling the sample holder and deposition substrates to 20 K and collecting another set of spectra. At 20 K, interstellar ice analogues composed of the most abundant volatile species in real interstellar ices (Boogert et al., 2015; Öberg 2016; McClure et al., 2023) were prepared on the substrates *via* the background condensation of gases and vapours: first, H_2O vapour from a de-ionised liquid sample that had been purged from gas contaminants *via* multiple rounds of the freeze-pump-thaw cycle was introduced into the chamber to deposit a layer of H_2O ice. This was followed by the co-deposition of a 1:1 mixture of NH_3 and CH_4, which was subsequently followed by the co-deposition of a 1:1 mixture of CO and CH_3OH. This methodology for the preparation of pyrimidines in the presence of interstellar ice analogues allows for a good mimic of the structure of actual interstellar ices, which are known to be segregated into a lower polar layer composed of hydrogenated heteroatoms (e.g., H_2O, NH_3, CH_4) and an upper apolar layer that develops after the catastrophic condensation of CO and its subsequent solid-phase hydrogenation to CH_3OH (Cuppen et al., 2024). The final result was therefore a series of layered H_2O:NH_3:CH_4:CO:CH_3OH ices having an approximate composition of 10:1:1:1:1 and thicknesses ranging between 1.25-2.19 μm deposited on top of interstellar pyrimidine analogues whose mid-infrared absorption spectroscopy could then be studied.

The exact composition of the deposited interstellar ice analogues was determined by spectroscopically measuring the molecular column densities N (molecules cm^{-2}) of each component using the equation:

$$N = \ln(10)\frac{\int_{\nu_1}^{\nu_2} \text{Abs}\, d\nu}{A} \quad (1)$$

where the integral in the numerator corresponds to the area of an infrared absorption band characteristic to a particular molecular species and A is the integrated strength constant associated with that band. The integrated band strength constants used to quantify the abundances of the different volatile ice components were taken from the work of Bouilloud et al. (2015) and are given in Table 1.

The molecular column densities of the pyrimidine nucleobases were measured in a similar way: for cytosine, this value was calculated by measuring the area of the so-called α-band, which is a defined series of absorption features over the 3500-2000 cm^{-1} range for which the integrated band strength constant is known (Vignoli Muniz et al., 2022). For thymine, the absorption feature at 940 cm^{-1} whose integrated band strength constant was recently quantified (Mejía et al., 2023) was used. Finally, for uracil, the area of the so-called β-band, corresponding to absorption features across the 3400-1890 cm^{-1} range for which the integrated band strength constant is known (Saïagh et al., 2015), was measured. The final column density ratios of the H_2O:NH_3:CH_4:CO:CH_3OH:pyrimidine interstellar ice analogues were 10:0.4:0.4:0.7:0.5:0.3 in the case of cytosine, 10:1:1:1:1:0.3 in the case of thymine, and 10:3:3:4:2:0.3 in the case of uracil. It was not possible to accurately determine the column density ratios of the three-component mixture (i.e., the interstellar ice analogue containing all three studied pyrimidines) using mid-infrared spectroscopy due to the overlap of the respective pyrimidine bands.

It is to be noted that a consequence of our interstellar pyrimidine analogue preparation technique is that the pyrimidines adopt a crystalline structure in a layer below the polar ice analogue layer. In reality interstellar pyrimidines are unlikely to be crystalline, and amorphous pyrimidines or pyrimidines intermixed with volatile ices could have been prepared through oven sublimation or effusive evaporation techniques (Materese et al., 2020; Gerakines et al., 2022). However, such techniques are not ideal when preparing interstellar ice analogues containing more than one pyrimidine species (as was desired in this study), since the heating of a 1:1:1 solid mixture of the three pyrimidine nucleobases of interest may have caused them to sublime at different rates, which would result in an ice analogue which is significantly enhanced or depleted in one or more of these nucleobases. Therefore, drop-casting represents a pragmatic yet chemically robust method of ensuring that the relative abundance of pyrimidines in our three-component mixture is as close to a 1:1:1 ratio as possible.

Table 1. Approximate positions of the bands (ν in cm^{-1}) used to identify and quantify the presence of volatile ice components and their associated integrated band strength constants (A in cm molecule^{-1}). Data taken from the work of Bouilloud et al. (2015).

Molecule	ν (cm^{-1})	A (cm molecule^{-1})
H_2O	3300 (ν_3)	1.5×10^{-16}
NH_3	1070 (ν_2)	1.6×10^{-17}
CH_4	1300 (ν_4)	8.0×10^{-18}
CO	2140 (ν_s)	1.2×10^{-17}
CH_3OH	1030 (ν_8)	1.1×10^{-17}

3. Results

3.1 Mid-Infrared Spectra of Pyrimidines at 300 and 20 K

The mid-infrared absorption spectra of crystalline cytosine, thymine, uracil, and the three-component mixture at 300 and 20 K are respectively shown in the top and bottom panels of Figure 2. The assignments of the bands observed in these absorption spectra, which are based on assignments made by previous studies (Susi and Ard 1971; Radchenko et al., 1984; Mathlouthi et al., 1986; Szczesniak et al., 1988; Nowak et al., 1989; Leś et al., 1992; Kwiatkowski and Leszczyński 1996; Colarusso et al., 1997; Szczepaniak et al., 2000; Singh 2008; Fornaro et al., 2014; Saïagh et al., 2015), are listed in Tables 2 and 3. It is possible to note a number of differences between the spectra of a particular pyrimidine nucleobase at the two investigated temperatures, which could suggest that the infrared spectra of these nucleobases in dense, quiescent molecular clouds (where temperatures typically range between 10-20 K) will differ to those of similar nucleobases in active star-forming regions (where temperatures may easily reach 300 K).

When comparing the mid-infrared spectra of cytosine acquired at 300 and 20 K, it is possible to note that the absorption bands present between 3500-2500 cm^{-1} (2.86-4.00 μm; corresponding to the ν NH_2 and ν CH vibrational modes) and 850-650 cm^{-1} (11.76-15.35 μm; corresponding to the γ NH_2, γ C=O, ring breathing, and β-ring vibrational modes) are noticeably sharper in the lower temperature spectrum (Figure 2). Additionally, a number of bands are apparent in the 20 K spectrum that are not visible in the 300 K spectrum. For instance, the double-peaked band at 791 cm^{-1} (12.64 μm) observed in the 300 K spectrum and assigned to the γ C=O mode appears as two distinct bands at 795 and 782 cm^{-1} (12.58 and 12.79 μm) in the 20 K spectrum, while the β_{rock} NH_2 mode at 1100 cm^{-1} (9.09 μm) is only visible in the 20 K spectrum. Such a phenomenon has been observed in previous studies (Rozenberg et al., 2004a; 2004b; Beć et al., 2019; Iglesias-Groth and Cataldo 2023), and is attributed to either the narrowing of the distribution of vibrational states at lower temperatures (Shen et al., 2003), or the suppression of the broadening originating from the interaction of fundamental modes with thermally excited low-frequency intermolecular deformation (i.e., libration) modes (Rozenberg et al., 2004a), or a combination of both.

Similar comparisons may be made between the mid-infrared absorption spectra of thymine acquired at 300 and 20 K (Figure 2). Once again, the bands in the 20 K spectrum are sharper than those in the 300 K spectrum; particularly those absorption bands between 3250-2600 cm^{-1} (3.08-3.85 μm) corresponding to the ν NH, ν CH, and ν CH_3 vibrational modes of thymine. Furthermore, a number of absorption features that are present in the 20 K spectrum adopt a different appearance or are entirely absent in the 300 K spectrum. For instance, bands in the 20 K spectrum at 1725 and 1373 cm^{-1} (5.80 and 7.28 μm) respectively attributed to the ν C=O and the γ CH_3 modes were not observed in the 300 K spectrum; nor was the band attributed to the ν-ring mode at 1452 cm^{-1} (6.89 μm). There are also regions in the 300 K mid-infrared spectrum of thymine where only one band is visible, whereas the corresponding regions in the 20 K spectrum are characterised by two or more absorption features. This is exemplified by the 850-800 cm^{-1} (11.76-12.50 μm) range, in which two absorption features (at 835 and 814 cm^{-1}; 11.98 and 12.29 μm) assigned to the γ NH mode of thymine are evident in the 300 K spectrum, but three bands (at 844, 818, and 800 cm^{-1}; 11.85, 12.22, and 12.50 μm) appear in this range in the 20 K spectrum.

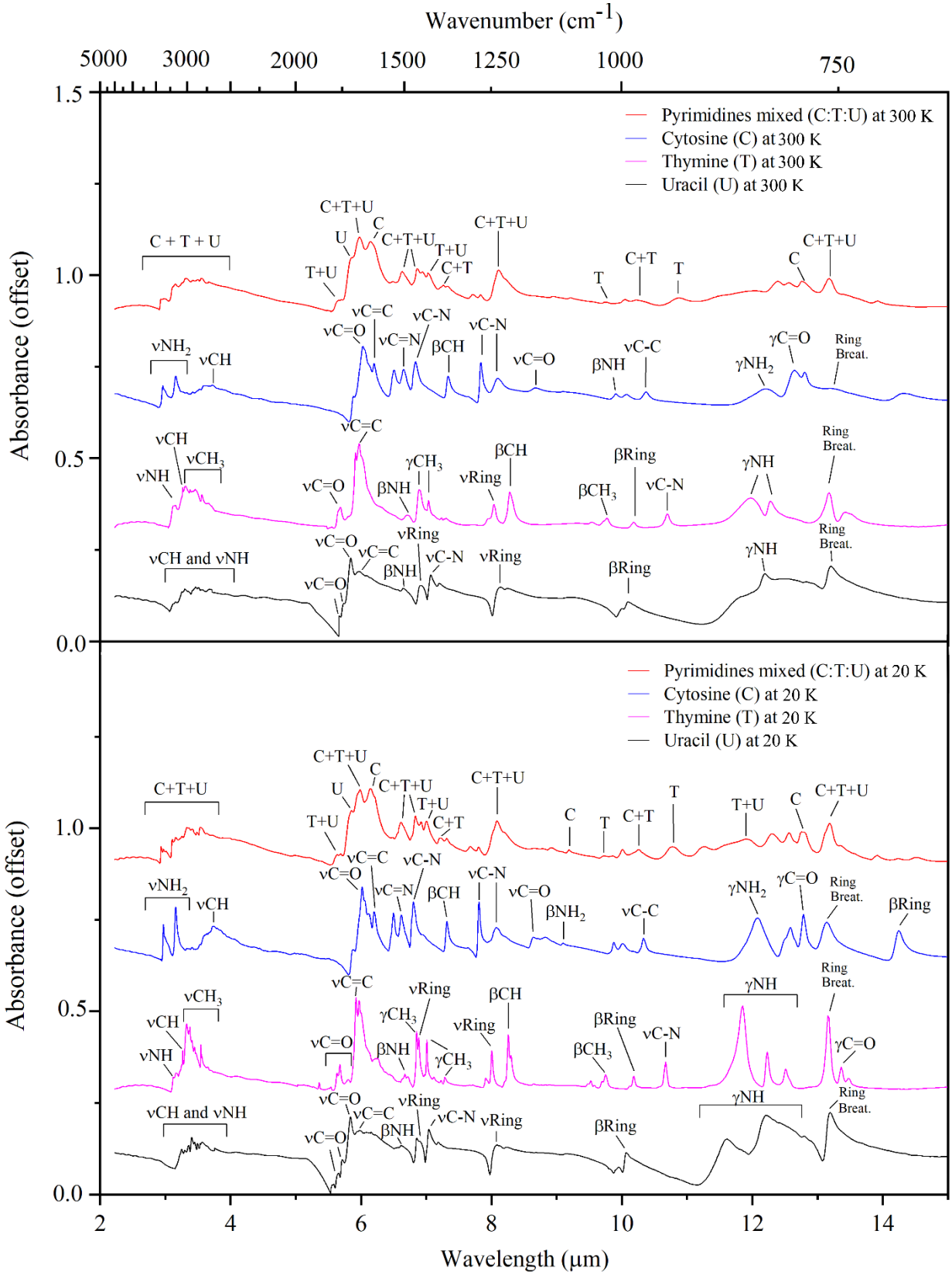

Figure 2. Mid-infrared absorption spectra of the neat pyrimidines cytosine (blue trace), thymine (pink trace), and uracil (black trace), and of the three-nucleobase mixture (red trace) at 300 K (top panel) and 20 K (bottom panel). Note that spectra are off-set along the vertical axis for clarity.

Table 2. Absorption band assignments for the mid-infrared spectra of neat cytosine, neat thymine, and neat uracil at 300 and 20 K.

Neat Cytosine				Neat Thymine				Neat Uracil			
ν (cm^{-1})		Assignment	Refs.	ν (cm^{-1})		Assignment	Refs.	ν (cm^{-1})		Assignment	Refs.
300 K	*20 K*			*300 K*	*20 K*			*300 K*	*20 K*		
3379	3367	ν_{asym} NH_2	*a*	3205	3205	ν NH	*h*	3077	3077	ν CH and ν NH	*i,j,k,l*
3167	3167	ν_{sym} NH_2	*a*	3062	3061	ν CH	*f,h*	3030	3031	ν CH and ν NH	*i,j,k,l*
2686	2676	ν CH	*a*	3022	3006	ν_{asym} CH_3	*h*	2978	2977	ν CH and ν NH	*i,j,k,l*
1660	1662	ν C=O	*a*	2961	2962	ν CH_3	*e,i,j*		2937	ν CH and ν NH	*i,j,k,l*
	1633	ν C=O	*a*	2932	2935	ν_{asym} CH_3	*h*	2886	2890	ν CH and ν NH	*i,j,k,l*
1613	1612	ν C=O	*a*	2892	2901	ν_{asym} CH_3	*h*	2855	2863	ν CH and ν NH	*i,j,k,l*
1537	1539	β NH_2	*a,b,c*	1759	1762	ν C=O	*e,f,i,j*	2801	2802	ν CH and ν NH	*i,j,k,l*
1503	1511	ν C=N and ν CN	*a*		1725	ν C=O	*e,f,i,j*	1772	1769	ν C=O	*e,i,j*
1463	1470	ν CN	*a,b,c,d,e*	1691	1688	ν C=C	*i,j*	1750	1750	ν C=O	*e,i,j*
1363	1369	β CH	*a,b,e,f*	1674	1674	ν C=O	*e,f,i,j*	1712	1713	ν C=O	*i,j,k,l*
1276	1280	ν CN	*a*	1491	1501	β NH	*h*	1674	1673	ν C=C	*k,l*
1235	1238	ν CN	*a,b,c,d,e*		1459	γ CH_3	*e,f,h,i,j*	1504	1512	β NH	*i,j,k,l*
1153	1157	ν C=O	*a*		1452	ν-ring	*h*		1459	ν-ring, ν CN, and β NH	*e,i,j,k,l*
	1100	β_{rock} NH_2	*a,b,c,e,g*	1421	1427	γ CH_3	*e,f,h,i,j*	1445	1445	ν-ring	*h*
1009	1012	β NH and β CH	*a*		1405	ν CN and β NH	*j*	1414	1420	ν CN and β NH	*e,i,j,k,l*
993	999	β CH	*a*	1383	1383	γ CH_3	*h*	1388	1394	ν CN, β CH, and β NH	*e,i,j,k,l*
964	968	ν CC	*a,b,c*		1373	γ CH_3	*h*	1228	1237	ν-ring	*k,l*
819	828	γ NH_2	*a,g*	1244	1249	ν-ring	*h*	1214	1216	ν CN, β CH, and β NH	*e,i,j,l*
791	795	γ C=O	*e*	1208	1211	β CH, ν CN, ν CC, and ν CCH_3	*e,f,i,j*	1001	1007	ν-ring	*l*
	782	γ C=O	*c,d,e*		1205	β CH	*h*	990	993	β-ring	*e,i,j,l*
757	761	ring breathing	*b,c,e*	1023	1025	ν-ring and β_{rock} CH_3	*e,f,h,i,j*		861	γ NH	*l*
698	702	β-ring	*a,c,d,e*	982	982	β-ring	*h*	820	819	γ NH	*h*
				935	937	ν CN	*h,i*	757	758	ring breathing	*e,i,j*
				835	844	?					
				814	818	γ NH	*h*				
					800	?					
				759	760	γ C=O and ring breathing	*e,f,h,i,j*				
				745	748	γ C=O	*e,f,h,i,j*				

References: (*a*) – Mathlouthi et al. (1986); (*b*) – Szczesniak et al. (1988); (*c*) – Nowak et al. (1989); (*d*) – Kwiatkowski and Leszczyński (1996); (*e*) – Fornaro et al. (2014); (*f*) – Szczepaniak et al. (2000); (*g*) – Radchenko et al. (1984); (*h*) – Singh (2008); (*i*) – Leś et al. (1992); (*j*) – Colarusso et al. (1997); (*k*) – Saïagh et al. (2015); (*l*) – Susi and Ard (1971).

Table 3. Absorption band assignments for the mid-infrared spectrum of a three-component mixture containing cytosine, thymine, and uracil at 300 and 20 K. Bracketed letters in the assignment indicate the nucleobase to which the absorption band is assigned: (C) – cytosine; (T) – thymine; (U) – uracil.

ν (cm^{-1})		Assignment	Refs.
300 K	*20 K*		
	3366	ν_{asym} NH_2 (C)	*a*
3170	3167	ν_{sym} NH_2 (C)	*a*
3074	3077	ν CH (U) and ν NH (U)	*i,j,k,l*
3018	3003	ν_{asym} CH_3 (T)	*h*
2927	2930	ν_{asym} CH_3 (T), ν CH (U), and ν NH (U)	*h,i,j,k,l*
2859	2868	ν CH (U) and ν NH (U)	*i,j,k,l*
2811	2827	ν CH (U) and ν NH (U)	*i,j,k,l*
1757	1759	ν C=O (T) and ν C=O (U)	*e,f,i,j*
1711	1710	ν C=O (U)	*i,j,k,l*
1673	1671	ν C=C (T), ν C=C (U), and ν C=O (C)	*a,e,f,i,j,k,l*
1626	1626	ν C=C (C) and ν C=O (C)	*a*
1543	1542	β NH (C)	*a,b,c*
1508	1512	ν C=N (C), ν CN (C), β NH (T), and β NH (U)	*a,h,i,j,k,l*
1457	1463	ν CN (C), β CH_3 (T), ν-ring (U), ν CN (U), and β NH (U)	*a,b,c,d,e,f,h,i,j,k,l*
1441	1445	ν-ring (U)	*h*
1422	1428	β CH_3 (T), β NH (U), and ν CN (U)	*e,f,h,i,j,k,l*
1377	1369	β CH (C) and γ CH_3 (T)	*a,b,c,e,g,h*
1278	1281	ν CN (C)	*a*
1233	1237	ν CN (C), ν-ring (U), ν-ring (T), β CH (T), ν CN (T), ν CC (T), and ν CCH_3 (T)	*a,b,c,d,e,f,h,i,j,k,l*
1087	1088	β_{rock} NH_2 (C)	*a,b,c,e,g*
1027	1029	β_{rock} CH_3 (T) and ν-ring (T)	*e,f,h,i,j*
	1015	β NH (C) and β CH (C)	*a*
995	999	β CH (C)	*a*
979	975	ν CC (C) and β-ring (T)	*a,b,c,h*
919	928	ν CN (T)	*i,j*
	840	γ NH (T) and γ NH (U)	*h,l*
807	813	γ NH (T)	*h*
796	796	γ C=O (C)	*e*
784	784	γ C=O (C)	*c,d,e*
759	758	ring breathing (C), ring breathing (T), and ring breathing (U)	*b,c,e,f,h,i,j*

References: (*a*) – Mathlouthi et al. (1986); (*b*) – Szczesniak et al. (1988); (*c*) – Nowak et al. (1989); (*d*) – Kwiatkowski and Leszczyński (1996); (*e*) – Fornaro et al. (2014); (*f*) – Szczepaniak et al. (2000); (*g*) – Radchenko et al. (1984); (*h*) – Singh (2008); (*i*) – Leś et al. (1992); (*j*) – Colarusso et al. (1997); (*k*) – Saïagh et al. (2015); (*l*) – Susi and Ard (1971).

In general, only a few differences are visible in the spectra of neat uracil acquired at 300 and 20 K (Figure 2). Once again, absorption bands are more clearly defined in the lower temperature spectrum compared to its higher temperature counterpart. One noteworthy difference between these spectra is the presence of a single band at 820 cm^{-1} (12.20 μm) ascribed to the γ NH mode at 300 K, which appears as two bands at 861 and 819 cm^{-1} (11.61 and 12.21 μm) in the 20 K spectrum.

The mid-infrared absorption spectra of the three-component pyrimidine mixture (i.e., simultaneously containing cytosine, thymine, and uracil) is shown as a red trace in Figure 2. In both the 300 and the 20 K spectra, it is possible to note the presence of absorption features that are unique to a particular pyrimidine nucleobase, as well as features arising due to a blending of the bands of cytosine and thymine (labelled 'C+T'), thymine and uracil (labelled 'T+U'), and of all three pyrimidine nucleobases (labelled 'C+T+U'). The general effect of this blending of individual nucleobase absorption features is that bands take on a broader profile and are thus more evident against the background continuum, with the most prominent in the 20 K spectrum being those bands at 1671, 1512, 1463, 1237, and 758 cm^{-1} (5.98, 6.61, 6.84, 8.08, and 13.19 μm).

An interesting result of our analysis is that there appears to be no significant shift in the positions of the pyrimidine nucleobase bands when neat compared to their corresponding positions in the three-component mixture in spectra acquired at 20 K. Indeed, the spectrum of the three-component mixture is very similar to the sum of the spectra of the three individual pyrimidines, as shown in Figure 3. This result mirrors our previous findings for the purine nucleobases adenine and guanine (Rosa et al., 2023), and could possibly be indicative of a more general non-shifting of the positions of the mid-infrared absorption bands of nucleobases when mixed with other complex organic molecules. However, it is to be acknowledged that this observation may also simply be a result of the nucleobase preparation technique used in this study (i.e., drop-casting), and that it is entirely possible that nucleobase band positions may shift if other preparative techniques, such as oven sublimation methods, are employed to prepare the interstellar nucleobase analogues. Future dedicated experimental and theoretical studies should therefore be performed to test whether the non-shifting of band positions observed in this study is dependent on the nucleobase preparation technique, or on other physical or chemical factors such as the polarizability of the nucleobase molecules or their relative abundance within the mixture.

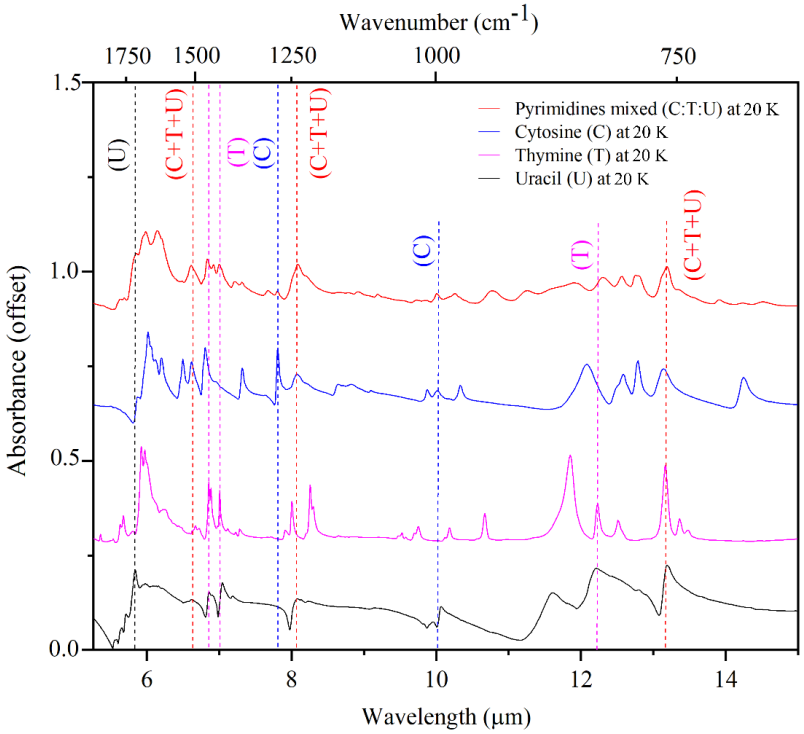

Figure 3. The mid-infrared absorption spectra of cytosine, thymine, and uracil as neat molecular ices at 20 K compared to that of the three-component mixture containing all the nucleobases. The vertical blue, pink, and black dashed lines respectively compare the positions of absorption bands solely attributed to cytosine, thymine, and uracil to those in the spectrum of the mixture. The vertical red lines compare the positions of blended bands in the spectrum of the mixture to those in the spectra of the individual pyrimidines. Note that spectra are off-set along the vertical axis for clarity.

3.2 Mid-Infrared Spectra of Pyrimidines in the Presence of Interstellar Ice Analogues at 20 K

The mid-infrared absorption spectra of a H_2O:NH_3:CH_4:CO:CH_3OH interstellar ice analogue having an approximate compositional ratio of 10:1:1:1:1 deposited on top of neat cytosine, neat thymine, neat uracil, and the three-component pyrimidine mixture are shown in Figure 4, with full assignments for each of the bands observed in the spectra provided in Tables 4 and 5. Our analyses of these spectra complement and extend the previous works of Rosa et al. (2024a; 2024b), who proposed the use of two common bands in the absorption spectra of neat cytosine, thymine, and uracil at 1246 ± 6 cm^{-1} (8.03 ± 0.04 μm; attributed to various vibrational modes) and at 761 ± 3 cm^{-1} (13.14 ± 0.05 μm; attributed to ring breathing modes) as possible mid-infrared

spectral signatures for the detection of interstellar pyrimidine nucleobases. The results of this present study demonstrate that the mid-infrared spectrum of a mixture of pyrimidine nucleobases (i.e., cytosine, thymine, and uracil) in the presence of an interstellar ice analogue contains many nucleobase bands that are obscured by the strong and intense absorption features of the volatile ice components (Figure 4). An analogous result was also observed in our previous work on purine nucleobases (Rosa et al., 2023).

Nevertheless, it is possible to distinguish a small number of pyrimidine mid-infrared absorption bands in the spectra of the three-component mixture in the presence of an interstellar ice analogue; these bands are highlighted in red in Figure 4. In particular, five absorption bands attributed to a blending of various cytosine, thymine, and uracil vibrational modes are evident at 1671, 1512, 1463, 1237, and 758 cm^{-1} (5.98, 6.61, 6.84, 8.08, and 13.19 μm). Since the main aim of the present study is the identification of pyrimidine mid-infrared absorption bands that could be potentially promising candidates for use in the detection of pyrimidine nucleobases in interstellar ices in a general sense, the remainder of this discussion will largely focus on these bands.

It is worth noting that, of these five bands, that at 1671 cm^{-1} (5.98 μm) is clearly distinguishable in the laboratory-generated spectra presented in Figure 4. However, it is likely that this band will not be as easy to distinguish in observational spectra of astronomical targets since it overlaps with the ν_2 mode of H_2O (Mifsud et al., 2022a). A similar argument may be made for the band at 1463 cm^{-1} (6.84 μm), which is partially obscured by the ν_{10} mode of CH_3OH (Gálvez et al., 2009). As such, we discount these two bands as potential mid-infrared spectral signatures of solid-phase interstellar pyrimidine nucleobases. Conversely, the three remaining bands at 1512, 1237, and 758 cm^{-1} (6.61, 8.08, and 13.19 μm) appear in less densely populated regions of the mid-infrared spectrum, and are therefore less likely to be obscured by the absorption features of ubiquitous icy volatiles in the observational spectra of astronomical targets. For this reason, we suggest that these bands (highlighted in Figure 5) may show some promise as spectral signatures for the detection of cytosine, thymine, and uracil in interstellar icy grain mantles, and thus certainly merit further investigation.

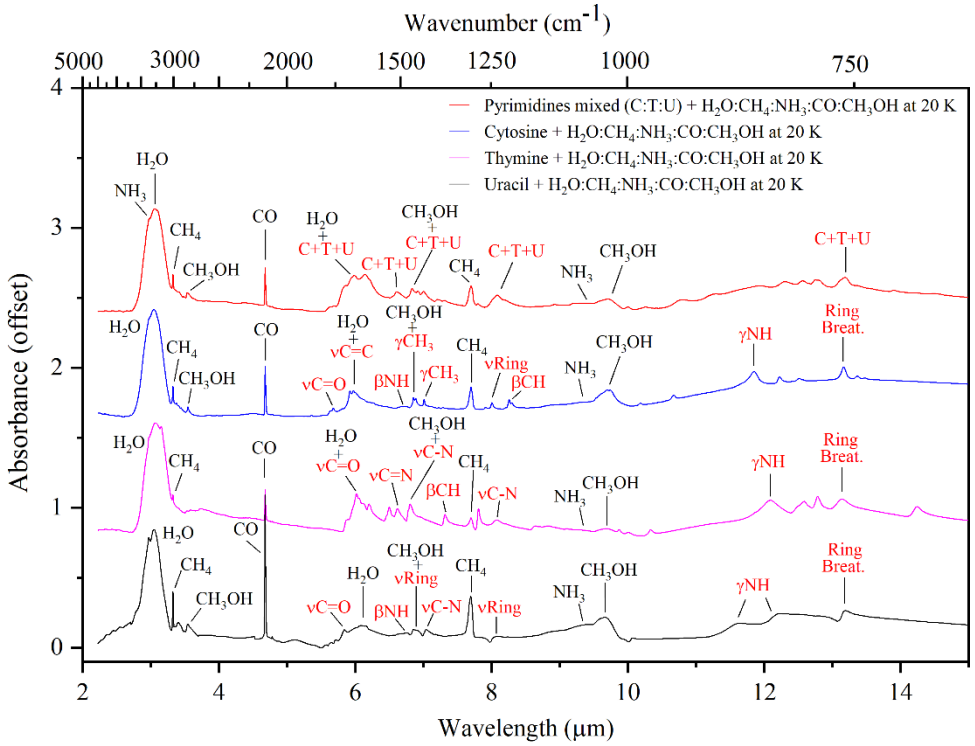

Figure 4. Mid-infrared absorption spectra of the pyrimidines cytosine (blue trace), thymine (pink trace), uracil (black trace), and the three-component pyrimidine mixture (red trace) in the presence of a H_2O:NH_3:CH_4:CO:CH_3OH (approx. 10:1:1:1:1) interstellar ice analogue at 20 K. Note that spectra are off-set along the vertical axis for clarity.

Table 4. Absorption band assignments for the mid-infrared spectra of neat cytosine, neat thymine, and neat uracil in the presence of a H_2O:NH_3:CH_4:CO:CH_3OH (approx. 10:1:1:1:1) astrophysical ice analogue at 20 K. Bracketed letters in the assignment indicate the nucleobase to which the absorption band is assigned: (C) – cytosine; (T) – thymine; (U) – uracil.

H_2O:NH_3:CH_4:CO:CH_3OH:cytosine		H_2O:NH_3:CH_4:CO:CH_3OH:thymine		H_2O:NH_3:CH_4:CO:CH_3OH:uracil	
ν (cm^{-1})	Assignment	ν (cm^{-1})	Assignment	ν (cm^{-1})	Assignment
3367	ν_{asym} NH_2 (C) and ν_3 (NH_3)	3284	ν_3 (H_2O)	3371	ν_3 (NH_3)
3259	ν_3 (H_2O)	3008	ν_3 (CH_4)	3282	ν_3 (H_2O)
3167	ν_{sym} NH_2 (C) and ν_3 (H_2O)	2822	ν_3 (CH_3OH)	3008	ν_3 (CH_4)
3009	ν_3 (CH_4)	2138	ν (^{12}CO)	2942	ν CH (U) and ν NH (U)
2138	ν (^{12}CO)	2091	ν (^{13}CO)	2827	ν_3 (CH_3OH)
1662	ν C=C (C) and ν_2 (H_2O)	1762	ν C=O (T)	2138	ν (^{12}CO)
1613	ν C=C (C) and ν_2 (H_2O)	1688	ν C=C (T) and ν_2 (H_2O)	2092	ν (^{13}CO)
1539	β NH (C)	1674	ν C=C (T) and ν_2 (H_2O)	1712	ν C=O (U)
1511	ν C=N (C) and ν CN (C)	1498	β NH (T)	1643	ν_2 (H_2O)
1470	ν CN (C) and ν_{10} (CH_3OH)	1459	γ CH_3 (T) and ν_{10} (CH_3OH)	1512	β NH (U)
1366	β CH (C)	1452	ν-ring (T) and ν_{10} (CH_3OH)	1459	ν-ring (U), ν CN (U), β NH (U), and ν_{10} (CH_3OH)
1299	ν_4 (CH_4)	1427	γ CH_3 (T)	1420	ν CN (U) and β NH (U)
1281	ν CN (C)	1405	ν CN (T) and β NH (T)	1300	ν_4 (CH_4)
1238	ν CN (C)	1384	γ CH_3 (T)	1239	ν-ring (U)
1157	ν C=O (C)	1373	γ CH_3 (T)	1065	ν_2 (NH_3)
1100	β_{rock} NH_2 (C)	1299	ν_4 (CH_4)	1034	ν_8 (CH_3OH)
1074	ν_2 (NH_3)	1249	ν-ring (T)	863	γ NH (U)
1031	ν_8 (CH_3OH)	1211	β CH (T), ν CN (T), ν CC (T), and ν CCH_3 (T)	817	γ NH (U)
1013	β NH (C) and β CH (C)	1205	β CH (T)	758	ring breathing (U)
999	β CH (C)	1063	ν_2 (NH_3)		
967	ν CC (C)	1032	ν_8 (CH_3OH)		
827	γ NH_2 (C)	982	β-ring (T)		
794	γ C=O (C)	937	ν CN (T)		
761	ring breathing (C)	844	γ NH (T)		
702	β-ring (C)	818	γ NH (T)		
		799	γ NH (T)		
		759	γ C=O (T) and ring breathing (T)		
		748	γ C=O (T)		

Table 5. Absorption band assignments for the mid-infrared spectrum of a mixture containing cytosine, thymine, and uracil in the presence of a H_2O:NH_3:CH_4:CO:CH_3OH (approx. 10:1:1:1:1) astrophysical ice analogue at 20 K. Bracketed letters in the assignment indicate the nucleobase to which the absorption is assigned: (C) – cytosine; (T) – thymine; (U) – uracil.

ν (cm^{-1})	Assignment
3368	ν_{asym} NH_2 (C) and ν_3 (NH_3)
3269	ν_3 (H_2O)
3008	ν_3 (CH_4)
2828	ν_3 (CH_3OH)
2138	ν (^{12}CO)
1759	ν C=O (T) and ν C=O (U)
1710	ν C=O (U)
1671	ν C=C (T), ν C=C (U), ν C=O (C), and ν_2 (H_2O)
1626	ν C=O (C), ν C=C (C), and ν_2 (H_2O)
1512	ν CN (C), ν C=N (C), β NH (T), and β NH (U)
1463	ν CN (C), β CH_3 (T), ν-ring (U), ν CN (U), β NH (U), and ν_{10} (CH_3OH)
1445	ν-ring (U) and ν_{10} (CH_3OH)
1428	β CH_3 (T), ν CN (U), and β NH (U)
1387	γ CH_3 (T)
1367	β CH (C) and γ CH_3 (T)
1299	ν_4 (CH_4)
1282	ν CN (C)
1237	ν CN (C), ν-ring (U), ν-ring (T), β CH (T), ν CN (T), ν CC (T), and ν CCH_3 (T)
1062	ν_2 (NH_3)
1032	ν_8 (CH_3OH)
1000	β CH (C)
974	ν CC (C) and β-ring (T)
925	ν CN (T)
838	γ NH (T) and γ NH (U)
812	γ NH (T)
796	γ C=O (C)
784	γ C=O (C)
758	ring breathing (C), ring breathing (T), and ring breathing (U)

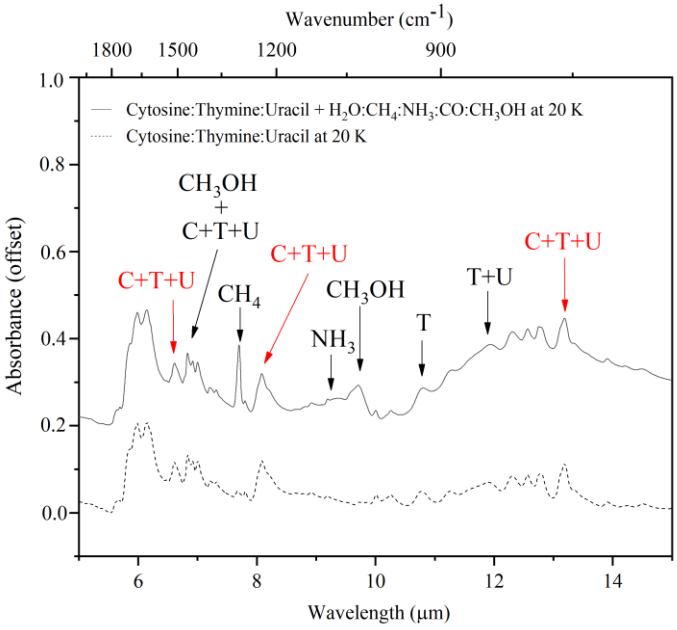

Figure 5. The mid-infrared absorption spectra of the three-component mixture containing cytosine (C), thymine (T), and uracil (U) in the presence of an astrophysical ice analogue at 20 K. Absorption bands suggested as potential signatures of interstellar solid-phase pyrimidines are highlighted in red.

4. Astrochemical Implications

Despite numerous attempts to identify the presence of pyrimidine nucleobases in the cold cores of dense interstellar clouds (Simon and Simon, 1973; Kuan et al., 2004; Charnley et al., 2005; Brünken et al., 2006), these molecules have yet to be identified in any extraterrestrial environment other than in meteorite fragments (Callahan et al., 2011; Burton et al., 2012; Oba et al., 2022). Given the known roles played by these molecules in biological and biochemical systems, their detection in astronomical environments represents a much sought-after result in contemporary astrochemical and astrobiological research. The most promising strategy towards detecting interstellar pyrimidines would be to search for their rotational emission spectrum; however, this would limit their detection to the gas phase. It is likely that pyrimidine nucleobases, like most complex organic molecules, are actually formed as a result of solid-phase chemical processes in interstellar icy grain mantles (Oba et al., 2019; Ruf et al., 2019). Due to the restriction of rotational motion in the solid phase, it is necessary to rely upon infrared spectroscopy as the detection technique for potential pyrimidine nucleobases in interstellar icy grain mantles.

However, one factor (among many; Rosa et al., 2023; 2024a; 2024b) contributing to the difficulty in identifying pyrimidine nucleobases in this way is the lack of mid-infrared spectroscopic data of these molecules in the presence of interstellar ice analogues at low temperatures. It is to be noted that cytosine, thymine, and uracil are structurally related molecules (Figure 1) that share common or similar abiotic synthesis routes and molecular precursors (Senanayake and Idriss, 2006; Saladino et al., 2005; 2012; 2015; 2016; Nuevo et al., 2009; 2012; 2014a; 2014b; Bera et al., 2010; Materese et al., 2013; Oba et al., 2019; Ruf et al., 2019); therefore, it is likely that the solid-phase interstellar synthesis of these molecules results in a mixture of pyrimidines, and so laboratory-generated spectra of such mixtures under conditions relevant to the interstellar medium are the most useful for the detection of pyrimidines in interstellar icy grain mantles. This therefore provides a strong motivation for our present study, as well as our previous analogous work on purine nucleobases (Rosa et al., 2023). Of course, our study is characterised by some limitations, including the crystalline nature of the nucleobase films.

As described previously, the preparation of crystalline pyrimidine nucleobases in this study by means of a drop-casting methodology was driven by experimental pragmatism. However, the crystallinity of our nucleobases does differ from the amorphous structure which is expected of nucleobases actually present in interstellar icy grain mantles. In general, the spectra of amorphous interstellar ice analogues typically exhibit broader and less structured absorption bands than do spectra of the same interstellar ice analogues in the crystalline phase (Mifsud et al., 2022a; Mifsud et al., 2022b). Comparison of our present spectroscopic results to those of other studies that considered the mid-infrared spectra of ice analogues containing a single pyrimidine nucleobase species in the amorphous phase (e.g., Materese et al., 2020; Gerakines et al., 2022) revealed that our spectra differ somewhat in that they exhibit moderately sharper absorption features as well as a number of instances of the Christiansen Effect (Prost 1973). This latter phenomenon is not observed in the mid-infrared spectra of astronomical targets (e.g., Boogert et al., 2015; McClure et al., 2023), and thus is likely not an impediment to the detection of potential spectral signatures of interstellar pyrimidine nucleobases. However, it is possible that the moderately sharper absorption bands observed in our study may overestimate somewhat the ease with which they may be detected in the spectra of astronomical targets.

Nevertheless, the spectroscopic data produced by the present preliminary study should go at least some way towards determining whether the detection of nucleobases in interstellar icy grain mantles is even possible. Our results indicate that, even when in the presence of abundant quantities of volatile interstellar ice components (i.e., H_2O, NH_3, CH_4, CO, and CH_3OH), the pyrimidine nucleobase bands at 1512, 1237, and 758 cm^{-1} (6.61, 8.08, and 13.19 μm) are distinguishable in laboratory-generated spectra and thus show some promise as spectral signatures for the detection of pyrimidines in interstellar icy grain mantles. This provides a useful starting point in the search for absorption features in spectra of icy astronomical targets that could be indicative of the presence of pyrimidine nucleobases. Of course, it is to be acknowledged that our study is preliminary in nature, and that it is necessary to check whether the three absorption bands identified as possible spectral signatures overlap

with those of other complex organic molecules. Although a rigorous and systematic analysis of this problem would go beyond the scope of this present study, we have assessed the mid-infrared absorption spectra of a number of nitrogen-bearing polycyclic aromatic hydrocarbons (N-PAHs) and formamide (Yang et al., 2020; Slavicinska et al., 2023; Wang et al., 2024) and have found no overlap of the absorption features of those molecules with the three pyrimidine bands identified in this study. Such a result is actually fairly significant, since these molecules are likely the major precursors to interstellar pyrimidines (Senanayake and Idriss, 2006; Saladino et al., 2005; 2012; 2015; 2016; Nuevo et al., 2009; 2012; 2014a; 2014b; Bera et al., 2010; Materese et al., 2013; Oba et al., 2019; Ruf et al., 2019). Future studies should, however, consider potential overlaps with other structurally or chemically unrelated organic molecules that are likely to be present in interstellar ices in higher abundances than pyrimidines (e.g., ethanol or acetone).

This is especially true given that any potential pyrimidine infrared absorption feature is very likely to be optically weak, and thus difficult to detect in astronomical spectra. Indeed, previous generations of infrared observatories such as the *Infrared Space Observatory* and the *Spitzer Space Telescope* were not able to unambiguously identify optically weak bands in the spectra of interstellar ices (see the works of Rosa et al., 2023; 2024a; 2024b for further information). Thus, the best chance of detecting such weak absorption features lies with the use of instruments with high spectral resolutions and signal-to-noise ratios. The Mid-Infrared Instrument (MIRI) aboard the *James Webb Space Telescope*, for instance, is able to capture very high-resolution observational data within relatively short exposure times: to compare, the MIRI has a resolving power which is about thirty times better than that of the Infrared Spectrograph (IRS) on board the *Spitzer Space Telescope*. As such, the *James Webb Space Telescope* currently represents the best opportunity to search for infrared spectral signatures of icy pyrimidine nucleobases and, indeed, has already successfully identified a number of complex organic molecules in interstellar icy grain mantles (Rocha et al., 2024).

It is also important to note that the three pyrimidine absorption bands at 1512, 1237 and 758 cm^{-1} (6.61, 8.08, and 13.19 μm) all possess calculated integrated molar absorptivity values greater than 4 km mol^{-1} (Table 6), as was recently reported by Iglesias-Groth and Cataldo (2023). The integrated molar absorptivity ψ is a spectrochemical parameter that indicates how strongly a molecular species absorbs or attenuates incident light across a defined wavenumber range. It is mathematically equivalent to the integral of the more commonly considered molar extinction coefficient ε across a defined wavenumber range and is related to the integrated area of an absorption band as measured from mid-infrared spectra as follows:

$$\psi = \frac{1}{lc}\int_{\nu_1}^{\nu_2} \mathrm{Abs}\, d\nu \qquad (2)$$

where l refers to the pathlength of the incident infrared light and c is the concentration of the absorbing molecule.

Table 6. Integrated molar absorptivity values for the absorption bands proposed as infrared spectral signatures of interstellar pyrimidines as calculated by Iglesias-Groth and Cataldo (2023). Note that band peak positions have an uncertainty of ±2 cm^{-1}. Bracketed letters indicate the nucleobase to which the absorption band is assigned: (C) – cytosine; (T) – thymine; (U) – uracil.

ν (cm^{-1})	λ (μm)	Assignment	ψ (km mol^{-1})
1512	6.61	ν C=N (C), ν CN (C), and β NH (T and U)	8.7 (C); – (T); 5.1 (U)
1237	8.08	ν CN (C) and ν-ring (T and U)	19 (C); 14 (T); 47 (U)
758	13.19	ring breathing (C, T, and U)	– (C); 8.7 (T); – (U)

Given that the three bands suggested herein to be potential infrared signatures of pyrimidine nucleobases in interstellar ices are all characterised by integrated molar absorptivities greater than 4 km mol^{-1} (Iglesias-Groth and Cataldo 2023), then in principle they should all be detectable in observational spectra acquired by the *James Webb Space Telescope* towards quiescent cloud and star-forming regions (McClure et al., 2023; Rocha et al., 2024). Moreover, these bands do not overlap with any of those proposed in our previous work as possible

spectral signatures for purine nucleobases (Rosa et al., 2023), and so it is possible, at least in principle, to distinguish between spectroscopic observations of purines and pyrimidines in environments known to host these molecules.

5. Conclusions

One of the central goals of astrobiology is the detection of molecules of biochemical or biological importance in extraterrestrial environments. Among the various key molecules that are yet to be detected, the purine and pyrimidine nucleobases are of particular interest due to their critical roles in genetic, evolutionary, and hereditary biochemical processes in terrestrial lifeforms. As such, the identification of nucleobases in interstellar environments would represent a major breakthrough in our understanding of the emergence of life in the cosmos, including on the early Earth. The previous detection of nucleobases in meteorite fragments supports the idea of their abiotic synthesis in extraterrestrial environments, and thus provides some motivation for their search in interstellar media, particularly in the icy grain mantles in which they are thought to be formed. However, such a search is complicated by the fact that the mid-infrared absorption spectra of icy interstellar targets are densely populated by absorption bands attributable to the volatile components of the interstellar ices, most prominently H_2O, NH_3, CH_4, CO, and CH_3OH. These absorption bands may overlap with or obscure those of lower concentration complex organic molecules, such as nucleobases, making the detection of the latter challenging.

In the present study, we have identified a set of three absorption bands at 1512, 1237, and 758 cm^{-1} (6.61, 8.08, and 13.19 μm) in the mid-infrared absorption spectra of a 1:1:1 mixture of cytosine, thymine, and uracil that remain evident even in the presence of an interstellar ice analogue composed of greater amounts of volatile ice species at 20 K. These three absorption bands all have sufficiently high integrated molar absorptivities that should, in principle, allow them to be detected by high-resolution and high-sensitivity instruments such as the *James Webb Space Telescope*. Although future dedicated spectroscopic studies focusing on the possible overlaps between these three pyrimidine absorption bands and those of other complex organic molecules likely to be present within interstellar ice analogues are required in order to fully understand and potentially exploit the utility of the former in detecting nucleobases in icy interstellar regions, our study nevertheless represents an important starting point in considering the possibility of such detections. Moreover, the results of this present study complement and extend those of our previous analogous work on purine nucleobases.

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Declaration of Interests Statement

All authors hereby declare that this research was performed in the absence of any interests (financial or otherwise) that may have biased its outcome.

Data Availability Statement

All data discussed herein will be made available to any interested party upon reasonable request of one of the corresponding authors.

CRediT Authorship Statement

Caroline Antunes Rosa: Conceptualisation, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing – Original Draft Preparation, Visualisation; ***Alexandre Bergantini:*** Methodology, Formal Analysis, Writing – Original Draft Preparation, Visualisation, Supervision, Funding Acquisition; ***Péter Herczku:*** Investigation; ***Duncan V. Mifsud:*** Investigation, Writing – Original Draft Preparation, Writing – Review and Editing; ***Gergő Lakatos:*** Investigation; ***Sándor T. S. Kovács:*** Investigation; ***Béla Sulik:*** Investigation, Resources; ***Zoltán Juhász:*** Investigation, Resources; ***Sergio Ioppolo:*** Resources; ***Nigel J. Mason:*** Resources, Supervision, Funding Acquisition; ***Claudia Lage:*** Methodology, Visualisation, Supervision

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