Chapter 5

HIGHLY SELECTIVE COMPLEXATION WITH 18-CROWN-6 ETHER SEQUESTERS PRIMARY AMINES IN SIMULATED TITAN AEROSOL FOR ENHANCED DETECTION AND STRUCTURAL ANALYSIS

5.1 Abstract

The aerosols present on Saturn's moon of Titan are proposed to contain molecules composed of carbon, nitrogen, and hydrogen. These aerosols have been simulated under laboratory conditions and found to be difficult to analyze due to their chemical complexity and the inclusion of a large number of different molecules. One particular expected functionality that has been the target of prior analyses are primary amines. Alkyl primary amines have been identified in prior work, but bulk primary amine identification and structural identification of said amines remains difficult. To not only identify a variety of primary amines but also obtain this additional structural information, a host-guest supramolecular complex with 18-crown-6 ether is utilized, enabling the identification of primary amines with minimal sample processing. The use of a MS^3 neutral loss method with an ion trap mass spectrometer allows for the unambiguous identification of primary amines and also the elucidation of some structural information. Compounds with nitrile functionality are identified, in addition to phenyl containing molecules. Both of these compound types present interesting implications for astrobiology and the complexity of primary amines possible in Titan's atmosphere.

5.2 Introduction

The Cassini-Huygens mission brought to light many interesting features of Saturn's moon Titan. While the lakes of methane and ethane[1], dunes[2], and river valleys[3] are all interesting, the atmosphere presents a more astrobiologically intriguing target. Titan's atmosphere is composed of nitrogen (90-98%) and methane (2-10%) with other trace gases.[4,5] The UV irradiation of this atmosphere produces molecules with various amounts of nitrogen incorporation that condense into aerosols and descend to the 95K surface.[6-8] Investigations of these aerosols by the Huygens lander found a large amount of chemical complexity, with even more complexity possible within molecules too large for detection with the mass spectrometer present on the mission.[5, 9] This has increased interest in the aerosols found on this moon and driven the desire to study these complex aerosols and characterize any prebiotic molecules found within.

To better study Titan's aerosols, simulants called tholins are made in the laboratory using a variety of different energy methods (discharge, cold plasma, UV), pressures, temperatures, and gas mixtures to simulate the atmosphere and induce chemistry.[10-25] The resultant brown to orange colored solid is then collected for subsequent analysis. These samples have been studied using a variety of methods including but not limited to nuclear magnetic resonance,[13, 14] gas chromatography,[16, 17, 26-29] UV and IR spectroscopy,[11, 15, 20, 30, 31] and electrospray mass spectrometry.[18, 21, 22, 27, 32] While all of these different methods help to provide useful information, most allow only for chemical functionality or chemical formulae to be determined. For methods that allow both pieces of information to be obtained along

with some structural identification there tend to be complications with particular functional groups or the identification of larger molecules within the mixture.

Amines are one functional group of astrobiological interest, due to their prevalence in common biological molecules such as amino acids. Amines are likely present in Titan aerosols and tholins but are difficult to detect with methods such as gas chromatography.[10] Aliphatic primary amines in tholins have been studied using a lab-on-a-chip capillary electrophoresis method with fluorescence detection.[33] The use of fluorescence necessitated the use of covalent derivatization conditions and matching of the resultant capillary electrophoresis peaks to readily available standards. While such a method is well suited to eventual in-situ mission applications, the need for standards necessitates complimentary methods for bulk identification prior to later characterization with mission applicable methodology. The desire to selectively identify a large variety of primary amines in these tholins makes this problem well suited to the use of host-guest supramolecular chemistry.

Supramolecular chemistry is a well-known area of physical organic chemistry, involving the formation of a larger subunit from two different molecules bound by non-covalent interactions such as hydrogen bonds or electrostatic forces.[34-36] The many interactions found within supramolecular chemistry often take inspiration from similar interactions found in biology.[37] There, supramolecular chemistry is responsible for the self-assembly of common structures such as the DNA double helix[38, 39] and the β-sheets of proteins.[40] Due to the prevalence and stability of these supramolecular assemblies,[38, 41-44] these types of interactions are utilized in multiple applications, from molecular recognition[45] to catalysis,[39] making supramolecular assemblies well suited for many analytical methods. One supramolecular assembly commonly used for analytical characterization is a host-guest

complex.[45-48] In these interactions a host molecule binds, in a reversible manner, with the smaller guest molecule containing a specific chemical functionality for which the host is sensitive.[43] These host-guest complexes are commonly seen in biology with enzymes and substrates, but are also used to assist in chemical synthesis and drug delivery.[37, 47] The versatility and selectivity of host-guest chemistry ability makes these complexes an attractive option for the analysis of tholins.

A major driver in the analysis of tholins are methods that enable the identification and characterization of target molecules within the mixture using minimal sample processing.10 Many other analytical methods used for such samples rely on covalent derivatization and subsequent chromatography.[49-51] These methods can increase experimental timescales and complicate the analysis if the necessary solvents or conditions are incompatible with certain compounds present, a known concern with the analysis of tholins considering their low solubility. Host-guest chemistry presents an alternative since they are typically sensitive to a particular functional group and can often be employed with milder conditions than covalent derivatization. The analysis of complex organic mixtures such as tholins usually relies on mass spectrometry, due to its ability to elucidate both chemical formula from the mass to charge ratio and structure by either conjunction with chromatography or the use of tandem mass spectrometry.[52-54] The host-guest complex used in the analysis of amines in tholins should take into account steps ease detection by mass spectrometry. The important factors in host selection are an increase in the mass range, especially if the proposed guest is small, facilitating the formation of a charged complex since mass spectrometry relies on detection of charged molecules, and that the host-guest complex is strong enough to survive the ionization process.

A selective host for primary amines is 18-crown-6 ether, shown in figure 5.1. This host is known to strongly complex to cations such as potassium, and also hydrogen bonds well with hydronium ion.[48, 55-60] This same hydrogen bonding has been used to complex 18-crown-6 ether to primary amines found in peptides and proteins,[61, 62] with complexation to secondary amines also observed.[48] This hydrogen bonding preserves the positive charge of the guest, making it well suited as a host for mass spectrometry.[55, 62] Additionally the crown ether complex has been characterized as stronger in the gas phase compared to solution. This phenomenon has been used to probe the solution phase structure of peptide-crown ether complexes during sampling by electrospray mass spectrometry.[61] 18-crown-6 ether has also been used with a variety of solvents to enable the separation of amino acids in capillary electrophoresis.[63] These factors and the ease with which this selective host-guest complex can be formed make it an ideal host for the study of primary amines in tholins.

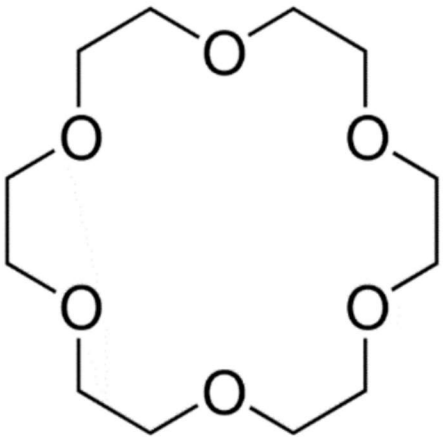

Figure 5.1. 18-crown-6 ether structure

In this study we probe the multiple different primary amines present within two tholin samples by 18-crown-6 ether complexation and analysis by MS/MS[64] and tandem MS[3] mass spectrometry, the latter employing a neutral loss (NL) scan instrumental method. This second method allows for the determination of both which molecules are bound to the crown ether and identification of some structure. The results from this study indicate that a large number of different molecules readily form complexes and primary amines comprise all of the guest molecules readily identified. The majority of those identified appear to follow set structural tendencies with some deviations. Since there are a large number of structural isomers possible within the material, identifications for the majority of compounds are limited to most likely chemical formulae. The breadth of data possible from this experiment would enable further identifications with increased sensitivity, resolution, or integration with chromatography. The range of primary amines identified by the 18-crown-6 ether host-guest complex show the suitability of the technique to enable the bulk identification of primary amines which has proven difficult with other methods. Additionally, the comparison of a new tholin to one that has undergone aging shows that while these amines are quite volatile compared to other species, they can still be identified even with decreased abundance. This has important implications for the identification of these primary amines in other tholin samples generated with lower yield methods or in other types of samples where the identification of low abundance primary amines is desired.

5.3 Experimental Methods

5.3.1 Titan Aerosol Simulants

The Titan aerosol simulants, or tholins, studied in this work have been well studied in other electrospray mass spectrometry experiments and their syntheses, described in detail in prior publications,[14, 18, 21] are summarized below. The new tholin was produced in a U-shaped glass reaction chamber held at 195K. A gas mixture of 95% nitrogen and 5% methane, with a flow rate of 6 atm L h^{-1} and a pressure of 1000 Pa, was exposed to a 60 Hz AC (10V peak to peak) discharge with a current of 30mA. After a typical run consuming 8 mol of gas, the solid was collected in a dry, oxygen free glove box and sealed until testing. Time and exposure between collection and testing were minimized for this sample.

The aged tholin was produced in a very similar manner, with a slightly modified apparatus.[14] A linear high vacuum stainless steel and glass reaction chamber held at 195K was filled with a mixture of 95% nitrogen and 5% methane. Gas flow during the 72 hour reaction time period was maintained to hold the chamber at 850 Pa. The reaction was accomplished by exposing the gas mixture to an AC electrical discharge with a current of 100 mA and an estimated discharge exposure time of 2 seconds. After synthesis the chamber was warmed to room temperature for 24 hours under vacuum and the solid collected under a nitrogen atmosphere. The collected solid has been exposed to and stored under ambient atmosphere in a dark freezer for two years prior to this study.

5.3.2 Materials and Methods

The dichloromethane used in the new tholin part of this study was dried using magnesium sulfate to ensure minimum water content in the solvent. 2mg/mL of new tholin was added to the dried dichloromethane, with no color change observed for the solution. 18-crown-6 ether was added to this solution to a concentration of 1-2mM. This mixture was electrosprayed on a Thermo LCQ Deca ion trap mass spectrometer. Instrumental parameters were modified to maximize the detection of the peaks of interest, resulting in a higher than normal 6.5 KV electrospray needle voltage. Two types of collision induced dissociation (CID) were used to study the sample. The first was in source CID, utilizing an innate setting within the LCQ with voltage settings between 0-12V. This applies a voltage to the octapole guide prior to trapping, allowing for fragmentation of any weak non-covalent bonds within the sample. The second was the traditional CID method, both MS/MS and MS^3 on selected peaks to confirm the formation of the complex and identify any structural possibilities.[64]

Different parameters were utilized for the study of the aged tholin. The anhydrous methanol (99.8%), anhydrous toluene (99.8%) and 18-crown-6 ether (99%) were all obtained from Sigma Aldrich. 3.8 mg of the aged tholin was dissolved in a 50/50 mixture of methanol and toluene with a 100 μM concentration of 18-crown-6 ether. While incomplete solvation was observed, the solution did display the color change expected with reasonable tholin solvation. This mixture was allowed to sit for at least 20 minutes to allow for maximum solvation prior to testing. The resultant mixture was electrosprayed using a Thermo LTQ-XL ion trap mass spectrometer. The instrumental parameters were tuned to ensure detection of the peaks of interest. A neutral loss (NL) method setting within the Xcalibur software was

used to perform MS3 NL scans, using CID, on the top 50 peaks. An isolation width of 2 m/z was used for both the MS2 and MS3 scans. Data were accumulated for 10 minutes, for which 13 separate cycles of 50 NL scans were performed. From these data, peaks targeted in at least 5 of the 13 cycles were used for subsequent analysis due to low intensity in the initial spectrum and the large number of observed peaks preventing analysis of all peaks of interest in each cycle.

5.4 Results

5.4.1 New Tholin

18-crown-6 ether has been characterized to show stronger interactions with guest molecules in solvents such as chloroform or dimethylformamide, or larger solvents like n-octanol.[48, 60] The increased stability of the complex in these solvents is proposed to be caused by the reorganization that occurs when the guest binds, which is readily accommodated with solvent environments that display decreased hydrogen boning to the oxygen atoms in the crown ether.[48] Additionally since 18-crown-6 ether readily binds to water, an easily dried or anhydrous solvent was necessary. These factors drove the selection of dichloromethane for this portion of the analysis, prioritizing complex formation over the solvation of the tholin sample.

The resultant electrospray mass spectrum can be seen in figure 5.2. The protonated 18-crown-6 and its water complex are the two most intense species, which is to be expected considering the high concentration of the crown ether and its ease of ionization. The species seen at lower mass display the characteristic pattern of peak envelopes expected of the

electrospray mass spectrum of tholin samples. This is seen with the consistent 14 m/z difference between the respective peaks. The higher mass species thus contain compounds complexed to the 18-crown-6 ether. The first series, identified by triangles in figure 5.2, corresponds to an amino nitrile series. These compounds have a general overall structure of $NH_2(CH_2)_xCN$, where the x=1-6 for this spectrum. Some of the compounds in this series are of astrobiological interest. The smallest in this series corresponds to aminoacetonitrile, identified by prior analyses of this sample and a prebiotic molecule that readily becomes the smallest amino acid glycine upon reaction with water.[27] The identification of multiple compounds in this series is important due to the inclusion of the nitrile group, believed to be a common functionality in Titan aerosols due to the abundance of hydrogen cyanide produced in the atmosphere.[10, 65] As such, the identification of compounds containing nitriles confirms that other nitrogen containing functional groups will not complicate the formation of the host-guest complex. The second series is not as readily identified but is based on an ion of 110 Da, with up to four additional methylenes. Since both series are regular it can be assumed that they are mostly linear, with the smaller ions necessitating linearity and the larger ions allowing for more variability. This linearity can be assumed since the region around the primary amine should have minimal steric hindrances to allow for the crown ether to move through multiple structural confirmations.

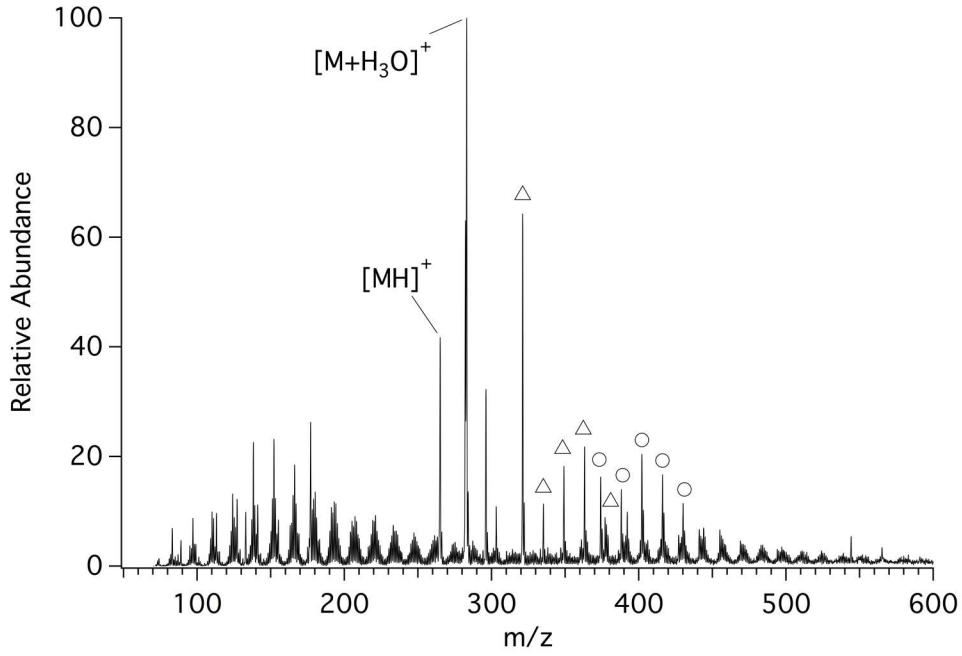

Figure 5.2. The overall mass spectrum for the 18-crown-6 ether and new tholin mixture. The 265 m/z species corresponds to protonated 18-crown-6 ether while the 283 m/z species corresponds to the water adduct. The triangles identify the amino nitrile series while circles correspond to the 110 Da series. Reproduced with permission from Hodyss 2006.[64]

Other possible guests for the 18-crown-6 ether need to be taken into account. It is easily seen from figure 5.2 that the crown ether readily binds with water, and it would also be possible to observe a complex with protonated secondary amines or imines. Both of these guests would display weaker binding compared to a primary amine guest. To take advantage of this difference, in source CID was used, shown in figure 5.3, with the intensities for each peak compared as the voltage was increased. It can been seen that the peaks corresponding to the two identified series do not readily drop off as voltage is applied, indicating they are strongly bound. The amino nitrile series appears to be the most strongly bound, considering the first peak in the series only drops to half intensity at 12V, while the peaks corresponding

to the 110 Da series drop off to half intensity by 8V. These data indicate though that the two series identified in figure 5.2 correspond to only primary amine complexes.

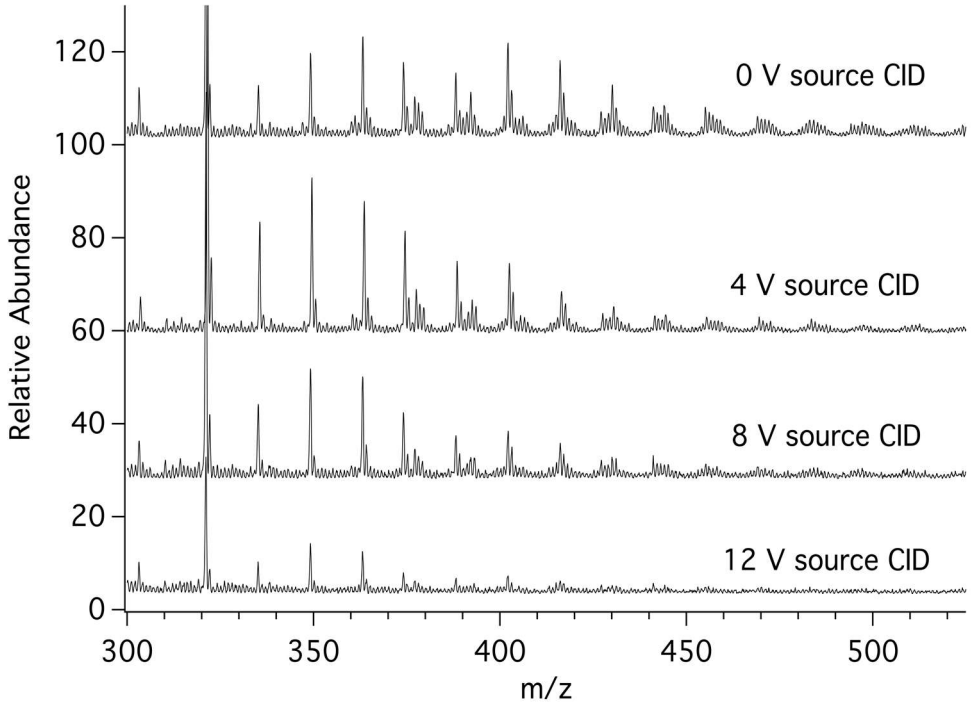

Figure 5.3. In source CID spectra for the 300-550 m/z region of the new tholin and 18-crown-6 ether sample. Reproduced with permission from Hodyss 2006.[64]

This initial experiment shows that the 18-crown-6 ether can successfully complex to primary amines within a tholin sample without competition from other protonated ions within the mixture. While these results are promising, the high discharge voltage is concerning for the analysis of the aged tholin. This could lead to decreased abundances of the molecules of interest and low ionization efficiency complicating their detection. As such,

experimental protocols were modified to facilitate a more thorough analysis of the aged tholin.

5.4.2 Aged Tholin

The selection of a different solvent for this experiment was an important first step due to multiple complicating factors. The solvents that enable the strongest complex formation with 18-crown-6 ether are known to not electrospray well, as seen in high electrospray needle voltage required in the analysis of the new tholin discussed above. Since a decreased amount of amines were expected in the aged tholin[20] a more efficient electrospray solvent was necessary to maximize detection. While most solvents are usable with 18-crown-6 ether, aprotic solvents are found be much more effective at dissolving the Titan aerosol simulants, which are well known to be difficult to solvate regardless of solvent type.[10] To allow for maximum solvation of the sample, increase host-guest complex formation, and still enable analysis by electrospray mass spectrometry, an anhydrous mixture of methanol and toluene was used. This solvent mixture was used in prior electrospray analyses of petroleum mixtures,[53] which contain high quantities of polar and non-polar compounds similar to tholins. The anhydrous nature of the solvents also limited the binding of the crown ether to water, decreasing competitive binding. The non-polar nature of the toluene is proposed to enable improved host-guest complex formation in solution, which is necessary to optimize the creation of the much stronger gas phase complex. While full solvation of the tholin was not observed, solvation was similar to that reported in electrospray

analyses of this material with a methanol/acetonitrile mixture,[21] enabling a reasonable comparison to prior work.

The overall mass spectrum for the aged tholin and 18-crown-6 ether mixture is shown in figure 5.4 below. The displayed mass range of this spectrum eliminates the intense protonated 18-crown-6 ether at 265 m/z and the 18-crown-6 ether and water complex at 283 m/z, both of which are evident in the new tholin spectrum shown in figure 5.2. A large number of different peaks are easily seen, with multiple different mass envelopes and peak intensities demonstrating the complexity of the material. While the preservation of a permanent charge would make the 18-crown-6 ether complexes the expected high intensity peaks, the high number of isomers combined with the aged nature of the tholin means that no complexes can be assumed and more investigation is required.

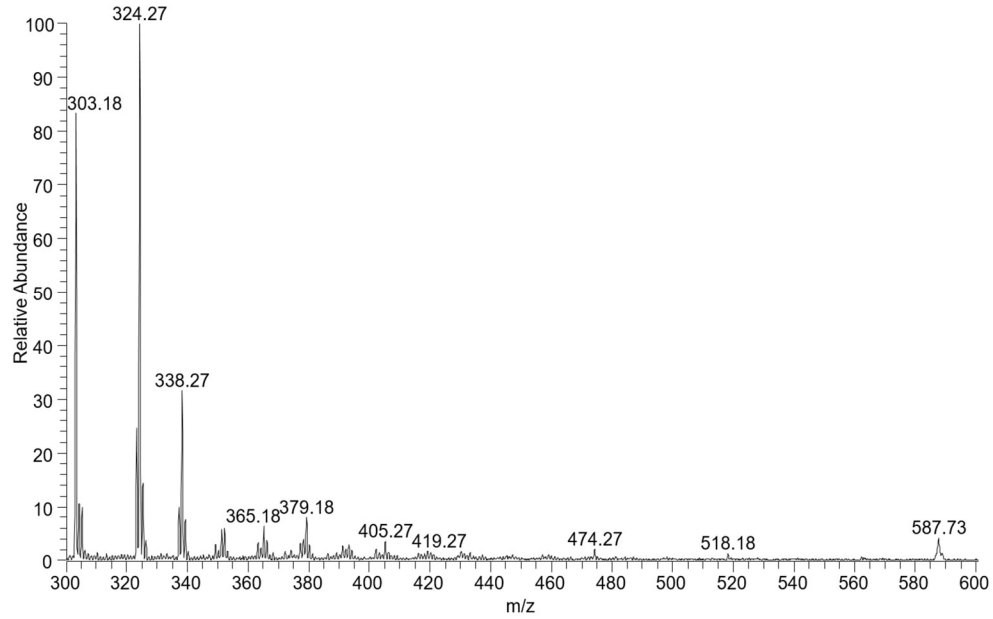

Figure 5.4. The overall mass spectrum for the 18-crown-6 ether and aged tholin mixture. The 303 m/z species corresponds to potassium cation complexed to the 18-crown-6 ether.

To properly identify crown ether complexes and obtain the greatest amount of structural information the use of tandem MS^3 mass spectrometry with neutral loss (NL) scans was utilized. A NL scan allows for the determination of complexes with the crown ether by scanning for crown ether loss (-265 m/z) from the parent peak using an automated method targeting the 50 most intense peaks in the overall spectrum. The initial scan for a NL from the parent is important due to the large number of different compounds likely within the isolation width (2 m/z) used. Not only is the complexed species and any isomers it contains targeted with this initial MS/MS scan, any molecules present in the tholin with the same nominal mass as the complex will also be fragmented. While this leads to complicated MS/MS spectra as seen in some figures below, the fragment corresponding to the NL species

is readily identified by this method. Additionally the preservation of the protonated amine makes the NL species the highest intensity peak observed in the MS/MS spectra for the majority of compounds examined, which further simplifies analysis.

If the NL of 264 Da, corresponding to 18-crown-6 ether, is detected, the initial fragment ion is further fragmented which provides structural information. In the case of primary amines the loss of 17 Da is seen, corresponding to the loss of the amine group as the figures below. Other fragments can lead to additional structural information, which can assist with identification. In the case of an ion trap mass spectrometer this identification can be complicated by the presence of isomers. The use of the NL scan helps to eliminate some of the isomers from the parent, but multiple isomers could still be isolated if each have the ability to complex to the crown ether. The structure of the complex itself can provide some insight for identifications, such as low steric hindrance around the primary amine being necessary as discussed above.

Taking the observed data into consideration, peaks reporting a loss indicative of a primary amine along with their proposed chemical formulae are shown in Table 1. These data show compounds that were either explicitly identified as showing a primary amine loss or, for >368 m/z and 374 m/z, based on the presence of the 265 m/z peak (protonated 18-crown-6 ether) in the NL loss scan. The fragments corresponded to the guest molecule for the latter compounds were either outside the mass range or low abundance in the NL scan spectra. Even without that extra confirmation, multiple different species can still be identified and analyzed. From an overall examination of the data it is interesting to note that the most intense species in the initial spectrum does not correspond to complexed species, as expected due to aging related losses from the tholin sample. This supports the use of a NL

mass spectrometry method to confirm the presence of complexes if a low abundance of primary amines is expected.

Complexed Species Mass (m/z)	MS^2 NL Peak	Most Likely Neutral Formula(e)	Proposed Structural Series Carbon Number		
			Alkyl Amine	Amino Nitrile	1 Degree Unsaturation
310	46	C_2H_7N*	2		
321	57	$C_2H_4N_2$*		1	
338	74	$C_4H_{11}N$*	4		
339	75	$C_3H_{10}N_2$*			
349	85	$C_4H_8N_2$*		3	
350	86	$C_5H_{11}N$*			5
351	87	$C_4H_{10}N_2$*			
352	88	$C_5H_{13}N$*	5		
353	89	$C_4H_{12}N_2$*			
363	99	$C_5H_{10}N_2$*		4	
364	100	$C_6H_{13}N$*			6
365	101	$C_5H_{12}N_2$*			
366	102	$C_6H_{15}N$*	6		
368	104	C_7H_5N, $C_4H_{13}N_3$*			
372	108	C_7H_9N, $C_5H_5N_3$			
374	110	$C_5H_7N_3$, $C_3H_4N_5$*°			
377	113	$C_6H_{12}N_2$		5	
378	114	$C_7H_{15}N$, $C5H_{11}N_3$			7
380	116	C_7H1_7N	7		
386	122	$C_8H_{11}N$			
391	127	$C_7H_{14}N_2$		6	
392	128	$C_8H_{17}N$, $C_6H_{13}N_3$			8
394	130	$C_8H_{19}N$	8		
401	137	$C_8H_{12}N_2$			
402	138	$C_7H_{11}N_3$, $C_5H_8N_5$°			
403	139	$C_8H_{14}N_2$			
404	140	$C_9H_{17}N$, $C_7H_{13}N_3$			
405	141	$C_8H_{16}N_2$		7	
406	142	$C_9H_{19}N$			9
416	152	$C_8H_{13}N_3$, $C_6H_{10}N_5$°			
417	153	$C_9H_{16}N_2$			
418	154	$C_{10}H_{19}N$, $C_8H_{15}N_3$			
419	155	$C_9H_{18}N_2$		8	
420	156	$C_{10}H_{21}N$, $C_8H_{17}N_3$			10
430	166	$C_9H_{15}N_3$, $C_7H_{12}N_5$°			
431	167	$C_{10}H_{18}N_2$, $C_8H_{14}N_4$			
433	169	$C_{10}H_{20}N_2$		9	

474	210	$C_{14}H_{27}N$, $C_{12}H_{23}N_3$			

Table 5.1. Data from the NL scan of the aged tholin and 18-crown-6 ether complex. Peaks from the overall mass spectrum, their NL scan mass, and most likely chemical formulae for the neutral guest molecule are shown. Those marked with an asterisk showed a 265 m/z peak in the NL scan but no MS3 scan of the guest were obtained. Structural series for the proposed formula are tabulated, with the number corresponding to the number of carbons present in the molecule for the alkyl amines, the number of methylene groups for the amino nitrile, and highest number of carbons possible for the unsaturated series. Those marked with a ° correspond to the 110 Da series identified in the new tholin spectrum shown in figure 5.2.

5.5 Discussion

5.5.1 New Tholin

Since the results shown above for the new tholin indicate the only complexes between 18-crown-6 ether and primary amines were observed for the identified series, further analysis could be attempted. One peak targeted for additional analysis was 416 m/z, the fourth in the 110 Da series. The MS/MS data, shown in the top spectra of figure 5.5, displays fragments corresponding to both the loss of 264 Da, the loss of the crown ether, and protonated crown ether. A peak corresponding to the water-18-crown-6 ether complex is also observed, which is believed to be related to complexation with water in the trap. The 152 m/z peak was further fragmented, displaying the loss of ammonia, indicating a primary amine, along with the loss of two different cyano groups. Since this sample has been studied in prior work, it is known to correspond to either $C_8H_{13}N_3$ or $C_6H_{10}N_5$.[21] From the observed fragments the compound contains a primary amine and a terminal cyano group which also allows for the loss of methyl cyanide. It is very possible that the other compounds within the 110 Da series also allow for

the loss methyl cyanide. While these are the same functionalities present within the amino nitrile series, the different binding affinity of the 110 Da suggest fundamental structural differences, likely related to the incorporation of a secondary or tertiary amine.

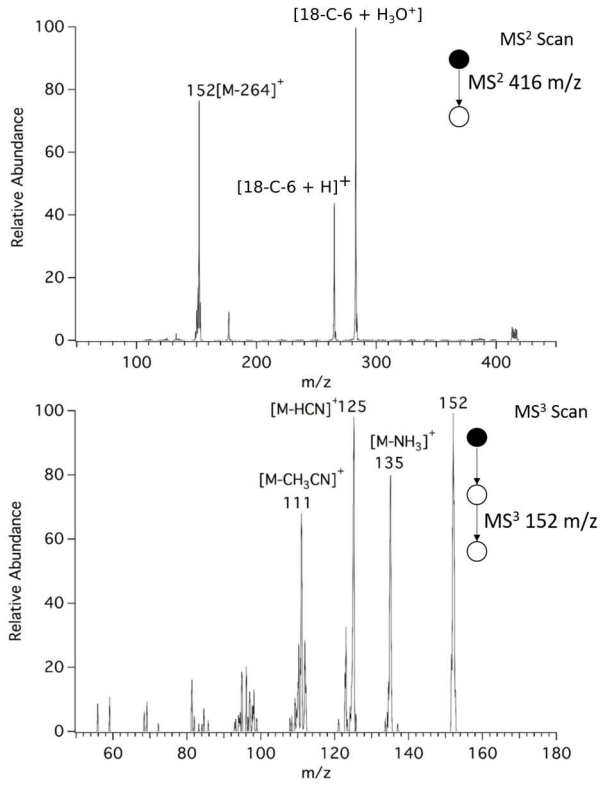

Figure 5.5 CID data for 416 m/z for the new tholin sample. Top: MS/MS spectrum of 416 m/z for the new tholin. Bottom: The MS3 spectrum for 152 m/z form 416 m/z. Reproduced with permission from Hodyss 2006.[64]

This example demonstrates that CID allows for dissociation of the complex, with MS^3 allowing for further fragmentation and an increased structural understanding. Other peaks within figure 5.2 could be identified, but it would require manual selection of each peak to screen for a complex with 18-crown-6 ether. The NL protocol utilized for the aged

tholins discussed above allowed for the automation of this selection process, overcoming the limitations from the manual selection of peaks. This NL method allowed not only for the increased identification of peaks, as shown in Table 5.1, but also the identification of multiple species as discussed below.

5.5.2 Aged Tholin

From the NL method data for the aged tholin, shown in Table 5.1, most likely chemical formulae can be proposed, and from these data four different structural series seem to dominate. The amino nitrile series was confirmed from the MS^3 scan, shown in figure 5.6, of the five methylene amino nitrile. There are four major fragments seen corresponding to losses from either the amine end of the molecule or the nitrile end, suggesting that the structure corresponds to an aliphatic amino nitrile with the formula $NH_2(CH_2)_4CN$, identifying the compound as 6-aminohexanenitrile, fitting with the assumption of a linear series discussed for the new tholin amino nitrile series. The loss of methyl and ethyl nitrile indicates that, while the 17 Da (NH_3) loss is expected for primary amines, the nitrile-containing losses will dominate over the loss of alkyl amines. This observation is important to the future interpretation of other peaks not examined in this work. While the other peaks corresponding to this series are not analyzed in detail, they are identified in Table 5.1. The observation of this series in the aged tholin is also encouraging since it is the main series observed in the new tholin sample. When comparing the series between the two samples it can be seen that the two methylene species is missing from the aged tholin, but three additional members of the series corresponding to 7-9 methylenes were newly identified.

The large difference in intensity for the same series in these two tholin samples demonstrates the impact that aging can have, which has been suggested by prior studies.[20]

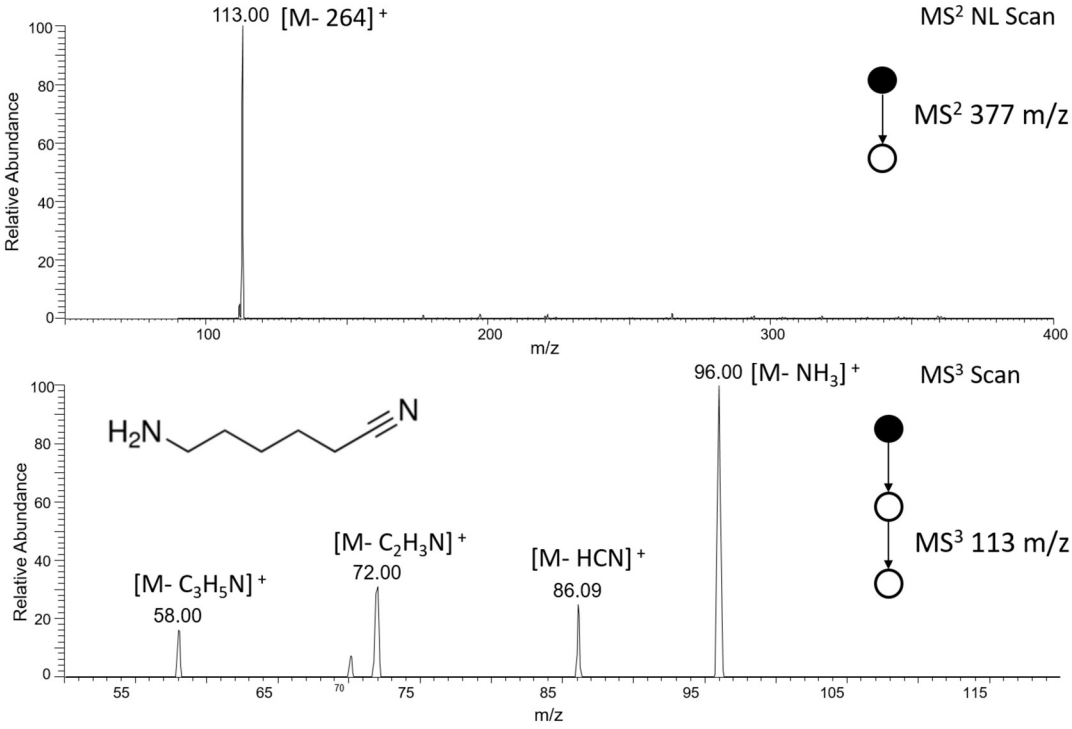

Figure 5.6. CID data for 377 m/z from the aged tholin sample. Top: MS/MS scan for 377 m/z showing the loss of the neutral 18-crown-6 ether (264 Da) as the major peak. Bottom: MS^3 scan for 113 m/z, showing losses indicative of an amino nitrile species.

A major series observed for the aged tholin unseen in the new tholin is the alkyl amine series, for which the smallest observed species corresponds to ethylamine. The seven carbon compound in this series was the smallest isolated for MS^3 (Not shown here), which presents complications. This is due to the large number of possible structural isomers as the number of carbons increase. As such, interpretation of these larger carbon MS^3 scans becomes difficult and outside the scope of the present work. The identification of a 17 Da

loss does cement these compounds as primary amines with minimal branching close to the amine. The lack of their explicit identification in this work demonstrates the need for chromatographic methods to identify these large carbon count alkyl amines; future study of these fragments with exact mass resolution could enable better identification of any branching present.[32]

The second newly observed series corresponds to a single degree of unsaturation and does not show a carbon count fewer than five within the suggested chemical formulae. This is suggestive of ring formation contributing to the unsaturation as opposed to a double bond, since no smaller compounds with regular unsaturation are observed and a five carbon ring would be the first stable ring for which a host-guest complex could form and 17 Da loss be possible. The increasing carbon counts likely correspond to additions to a six carbon ring. The seven carbon compound in this series was able to be examined by MS^3, as is shown in figure 5.7. This spectra shows the loss of a primary amine which is expected, but two other losses that are more difficult to interpret. The loss of 29 Da shown by the 85 m/z peak is suggestive of a methylimine loss, while the 41 Da loss from the 73 m/z peak matches best with the loss of acetonitrile. While the methylimine is possible from double bond formation during fragmentation of the ring, the acetonitrile loss is not possible with a single nitrogen structure that still complexes to a primary amine. The nitrile presence suggests more nitrogen incorporation. There are no stable structures with two nitrogen atoms that would allow for the observed losses, indicating that at least three nitrogen atoms are necessary to produce all of the fragments seen.

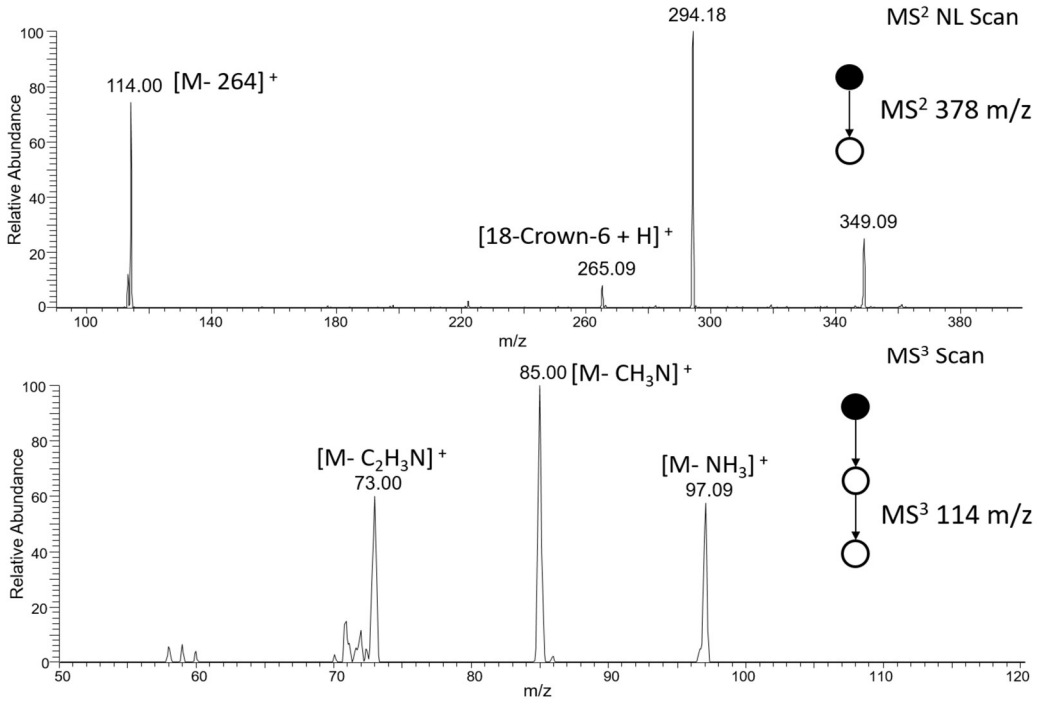

Figure 5.7. CID data for 378 m/z from the aged tholin sample. Top: MS/MS scan for 378 m/z showing the loss of the neutral 18-crown-6 ether (264 Da) as the second most intense peak and the protonated 18-crown-6 ether. Bottom: MS^3 scan for 114 m/z, showing losses of ammonia, methylimine, and acetonitrile. These losses are suggestive of multiple structural isomers.

The loss of both a primary amine and methylimine is suggestive of another possibility: the observation of more than one structural isomer within the same neutral loss scan. This would allow for the complexation of one compound, a cyclic primary amine that readily loses 17 Da and produces the observed 97 m/z and 85 m/z peaks, and a different isomer that contributes to these peaks while also producing the 73 m/z fragment. The second isomer could contain a nitrile and a methyl substituted secondary amine. This would help to explain the methylimine loss being the most intense fragment, since it could be possible from both proposed isomers. The presence of two isomers is further supported by the observation

of both the protonated 18-crown-6 ether and the loss of the crown ether in the NL scan, as opposed to the observation of only the NL species seen in the amino nitrile spectrum in figure 5.6. The observation of both peaks suggests that the different isomers seen have different binding affinities to the crown ether. This would follow from one isomer containing a substituted secondary amine, since secondary amines have lower proton affinities compared to primary amines. The large number of different possibilities for these isomers precludes identification but suggests that the unsaturation observed for the primary amine loss could relate to either a ring formation or higher nitrogen inclusion. This higher degree of nitrogen incorporation also helps explain why unsaturation is observed only for higher mass species. The possibility of both isomers being contained in this series is accounted for in Table 5.1 and shows the care necessary in these identifications.

While the possibility of isomers presents difficulties for some identifications, others are simplified by structural possibilities. This is most obvious for the NL and MS^3 scans of 372 and 386 m/z, shown in figure 5.8 a and b respectively. Both MS3 scans show a peak at 91 m/z, which most likely corresponds to a toluene $[M-H]^{\bullet+}$ radical cation, due to its stability and observation in the MS/MS spectra of other substituted benzene molecules. This would make the complex at 372 m/z correspond to benzyl amine by comparison with prior reported MS/MS data. Taking the 91 m/z in the MS^3 scan of 386 m/z to be the same toluene cation, the 105 m/z fragment would also correspond to another substituted benzene molecule. Comparison with other MS/MS data can also provide a most likely identification of phenylethylamine for this second compound. These data show that with distinctive fragments, identifications can be made even if multiple isomers are possible.

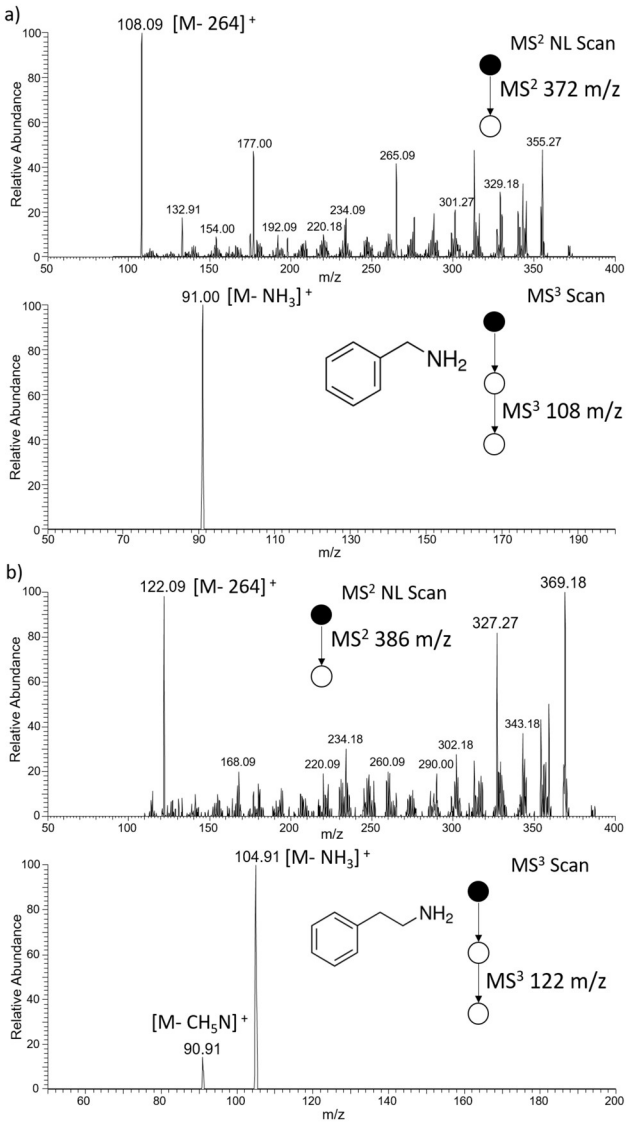

Figure 5.8. CID data for benzene containing species from the aged tholin sample. a) *Top*: MS/MS scan for 372 m/z showing the loss of the neutral 18-crown-6 ether (264 Da) as the most intense peak with multiple other fragments also visible. *Bottom*: MS^3 scan for 108 m/z, showing loss of ammonia and a fragment indicative of a toluene radical cation. b) *Top*: MS/MS scan for 386 m/z showing the loss of the neutral 18-crown-6 ether as the second most intense peaks, with another loss from a non-complexed 386 species as the dominant fragment. *Bottom*: MS^3 scan for 122 m/z, showing loss of ammonia from one fragment and a second fragment from methylamine loss also matching a toluene ion. Both of these fragments are assigned to benzene containing species.

The identification of two substituted benzene molecules is ideal for the characterization of tholins. These types of amines would be expected on Titan due to the prior observation of benzene in Titan's atmosphere.[8, 66] The production of two different substituted benzene molecules with a methylene difference between then suggests more complex chemistry for primary amines is possible and possibly favored. This second possibility is further supported by the lack of aniline, which was expected from ammonia addition to a phenyl radical. Benzyl radicals are more stable than phenyl radical and thus may be more readily synthesized and available for reactions with small amines. Since other substituted benzene molecules were not identified with this method when looking for the 91 m/z fragment, there may be quenching characteristics to the chemistry that truncate subsequent reactions. This could be due to consumption of smaller amines and ammonia in the synthesis of the many other primary amines observed in the material. Benzene is much more complicated than the other subunits observed here, and the longer timescale for its synthesis could be a limiting factor since ammonia may already be consumed in other reactions and not available in a large enough scale. While these observations would need to be confirmed, this does show that the 18-crown-6 ether complex can readily form with more complex primary amines.

The final observation true for both the new and aged tholin samples was the lack of any doubly complexed species. Since both species display the amino nitrile series and the 110 Da series it can be assumed that this is also a fundamental characteristic common between the tholins. This suggests that, contrary to what might be expected from such a complex mixture, compounds with two sterically available primary amines are not readily

produced during tholin synthesis. Some of the possible reasons for this observation include increased reactivity of amines within the plasma, or compounds with two primary amines being more volatile and readily lost. The most likely possibility is that the high energy of the plasma contributes to an increased abundance of other functional group which decreases the probability of forming a compound with two sterically available primary amines. These high energy processes may also convert a second primary amine to a secondary or tertiary amine, precluding their detection.

The data presented here show that the 18-crown-6 ether host-guest complex with primary amines is effective for the identification of various primary amines within the tholin sample without the need for extensive preprocessing. The in source CID method combined with the MS^3 NL method allow for the unambiguous identification of primary amines as the favored guest, confirming that the complex is not readily formed with secondary amines or imines. The MS^3 NL method provides much needed structural information. Production of primary amines has prebiotic significance, but the variety of primary amines observed in this experiment demonstrates that they may be a more common substituent than expected.

While the primary amines observed in the aged sample are lower in intensity and thus not present in high quantities compared to the new tholin, they also remained stable under atmospheric storage for two years. This suggests that enough primary amines are produced in stable enough confirmations to allow for detection after atmospheric degradation and evaporative losses. Combined with the observation of two regular series in the new tholin, it can be assumed that primary amines with a range of complexity likely comprise a reasonable amount of molecules within the tholins, making their identification necessary for proper characterization of the material.

Identification of complexes for which two different isomers are possible presents an additional difficultly, since the crown ether complex does not discern beyond a sterically available primary amine. More specific identification would require increased sensitivity or mass resolution. These studies would need to be combined with MS^n data to allow for structural identification, for which these data may provide a starting point upon which to base that future analysis. Another possibility would be the incorporation of the 18-crown-6 ether with high-performance liquid chromatography as a post column reagent. This would allow for the identification of different structural isomers assumed to be present for the more complex MS^3 data in this study. The initial 18-crown-6 either complexation does present a complement to other analytical methods, allowing for the unambiguous identification of a primary amine functionality and the precise identification of some molecules. These data provide interesting insights into the prevalence of primary amines within tholins and the difficulties in their characterization.

5.6 Conclusions

A supramolecular host-guest complex for the detection of primary amines in two tholin samples representative of Titan's atmosphere has been presented using an 18-crown-6 ether host. The complex was observed for various primary amines within each sample without extensive sample processing. The complexes formed were confirmed as primary amines and a MS^3 NL method allowed for additional structural information to be obtained. From the available data possible chemical formulae are proposed and some compounds identified, including a compounds with both amine and nitrile functionalities along with

substituted benzene molecules. Both of these have important implications for Titan's atmospheric chemistry due to their expected occurrence on Titan and, in the case of the aminoacetonitrile, possible astrobiological significance. The significant presence of primary amines in the aged tholin show that, while a new tholin sample is necessary for high intensity peaks, primary amines in tholins are produced in high abundance and are relatively stable. These data show that this supramolecular complex presents a complementary analytical method for the identification of primary amines within tholins and establishes a solid foundation for future analyses.

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