Electronic Supplementary Information for

Heterogeneous Cation Induced Clusters formed at Micro-Droplets Surface

Contents

1 Chemicals and Experimental Details

1.1 Chemicals

Trimethylamine (TMA), ammonia (30% aqueous solution), ammonium acetate, pentanoic acid, decanoic acid, pentadecanoic acid and dimethylamine are obtained from Sinopharm Group (Beijing, China). Thymine and imidazole are obtained from Sigma-Aldrich (Shanghai, China). All these chemicals are of analytical grade. Methanol (M) is obtained from Fisher Scientific (Pittsburgh, PA, USA), which is of HPLC grade. Pure water (W) is purchased from Wahaha Company (Hangzhou, Zhejiang, China). All these reagents or solvents were used as received. Solutions were freshly prepared in 1:1 (v/v) methanol/water solvent and then used without long time stocking.

1.2 Multichannel ESI Array

The home-built multichannel ESI array, previously named as "Multichannel Rotating Electrospray Ionization, MRESI" has been described elsewhere.[1] Generally, as shown in Scheme S1, three ESI emitters were evenly located at the circle plate in front of the MRESI. High voltage of 6.5 kV was applied to induce ESI sprays to post-ionize the neutral plume. The injection rate was 20 μL/h. The rotating axis of the MRESI is hollow, into which a neutral desorption (ND) or neutral spray (NS) tube can be inserted. The injection speed of the three emitters can be controlled by a syringe pump.

In our previous work, we have evidenced that the rotation of ESI emitters can equalize sampling rate of each channel. While in this work, since all three channels are always infused with the same solution, equalizing is not needed. So all the experiments were conducted under static condition.

Mass spectrometry conditions: MS experiment was done using a Thermo Finnigan LCQ Advantage MAX ion trap mass spectrometer (San Jose, CA, USA). The voltage of tube lens offset was set at -80 V, and the inlet temperature was 100 °C. All mass spectra were acquired in positive mode with 3 microscans and were recorded by the instrument software (Xcalibur version 1.4 SR1).

1.3 Dual NanoESI via Theta Capillary Tips

Dual pipettes were fabricated from quartz theta capillaries (1.2 mm outer diameter, 0.90 mm inner diameter) using a laser-based P-2000 pipette puller (Sutter Instrument Co.) Each dual pipette was used only once to avoid cross contamination. A platinum wire (O.D. 250 μm) was inserted in either one of the barrel. High voltage of 1.5 kV was applied on the Pt wire to induce nanospray. Details of the experimental procedure can be obtained from our previous work.[2]

1.4 Neutral Desorption and Neutral Spray System.

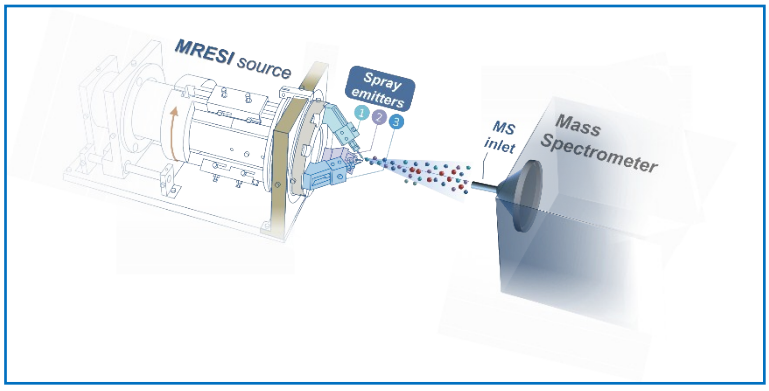
Neutral Desorption[3] (ND): A small glass bottle as shown in Fig 1a was used. It has an inner diameter of 3 cm, with a height of 6 cm. Pure nitrogen gas (Haike Yuanchang, Beijing, China) was used to desorb analytes from liquid or solid samples. The gas transferring rate was 8 L/min (which equals to linear speed of about 1100 m/s).

Neutral Spray (NS): A home-built ESI configuration[4] was applied as the NS system, but no high voltage was applied. Nitrogen gas was used as the nebulizing gas, with the transferring rate of 2 L/min. The linear speed of the nebulizing gas was lower than that of sound, avoiding sonic spray ionization[5]. The injection rate was 60 μL/h, to make in conditions 1 to 4 (Fig 4a), each reagent of the same concentration was introduced into the ionization zone.

1.5 The Analyses of Fish Meat

Three kinds of fishes (Crucian, Cod and Dory) were purchased very early in the morning from a local market. Samples for analyses were cut into 3g pieces, spiked with ammonia (0.1% v/v) and put into ND bottle. Thymine solution (0.2 mM) were infused in the three ESI emitters. When the desorbing gas, high ESI voltage and MS analyzer were turned on, the MS signals can be obtained.

The calibration curve was made by spiking increasing amount of TMA on fresh Crucian fish meat pieces. The signal intensity of m/z 1110 on these Crucian fresh pieces without TMA addition was negligible. The obtained LOD of TMA is 0.2 ppm, with the S/N ratio of 3.



Scheme S1 Illustrations of the MRESI instrument used in this work. Details of constructing this device can be found elsewhere[1].

2 Supplementary Experimental Results and Discussions

2.1 Fragmentation of m/z 1110

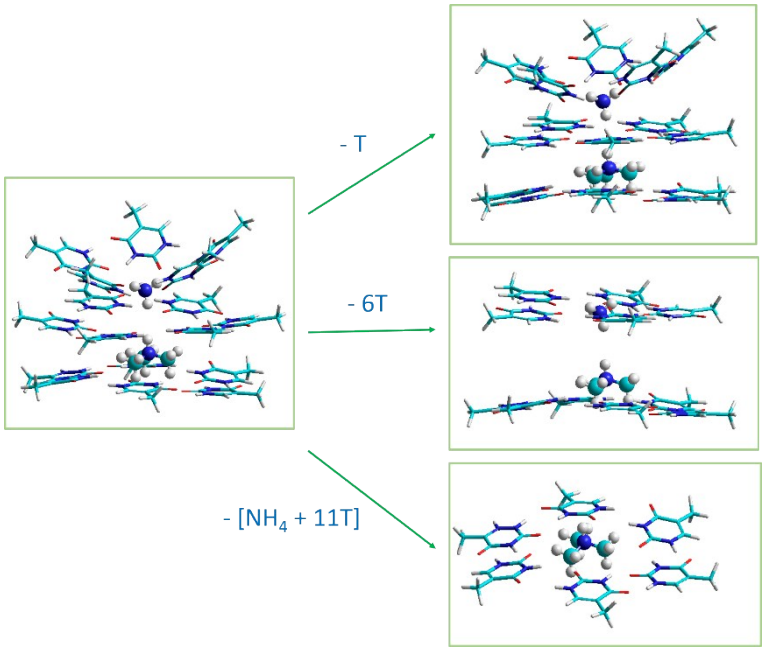


Fig S1 Proposed Fragmentation Pathways of the HeteroCIC m/z 1110. The first pathway is losing one thymine (T) from the "waist circle". The second pathway is losing the whole "waist circle". In the third pathway, the fragment of $[TMA+T_6]^+$ can be obtained.

2.2 Dual NanoESI

In dual nanoESI via theta capillary tips, two solutions from the tow barrels in the pipette can *in situ* mix at the Taylor cone in liquid phase. The life time of the generated micro-droplets is in the range of milliseconds. As shown below, HeteroCIC cluster cannot form in this condition.

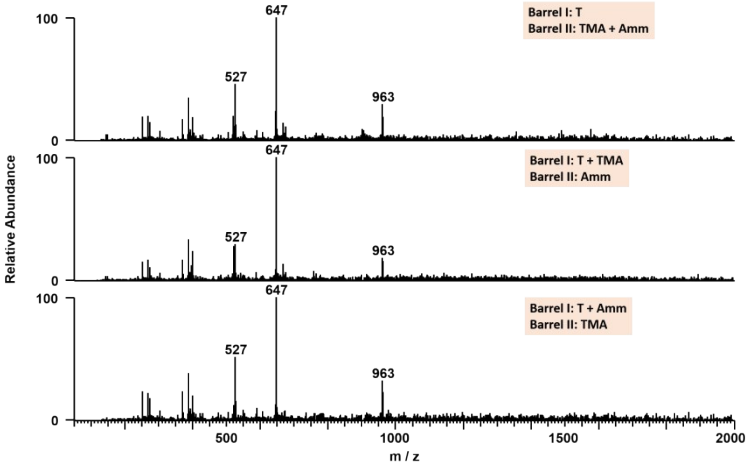


Fig S2 Dual nanoESI mass spectra. (a) Barrel I: thymine solution (0.2 mM), Barrel II: mixed solution of TMA (0.6 mM) and Ammonia (0.1 % v/v). The same concentrations of each reagent were applied in (b) and (c). (b) Barrel I: mixed solution of thymine and TMA, Barrel II: Ammonia. (c) Barrel I: mixed solution of thymine and ammonia, Barrel II: TMA.

2.3 The Influence of Surfactant on HomoCIC and HeteroCIC Formation

Ammonium salts were obtained by mixing ammonia with corresponding carboxylic acids. 0.01 mM Ammonium salt was added in the ESI solution to affect the clusters formation process. The "in" liquid cluster refers to the HomoCIC cluster m/z 963, $[2NH_4+T_{15}]^{2+}$. While the "on" liquid cluster refers to the HeteroCIC cluster m/z 1110, $[TMA+H+NH_4+T_{17}]^{2+}$. As shown below, the surfactant has more intense effect on inhibiting the HeteroCIC formation. This evidence can support our proposal that the HeteroCIC cluster is formed at micro-droplets surface.

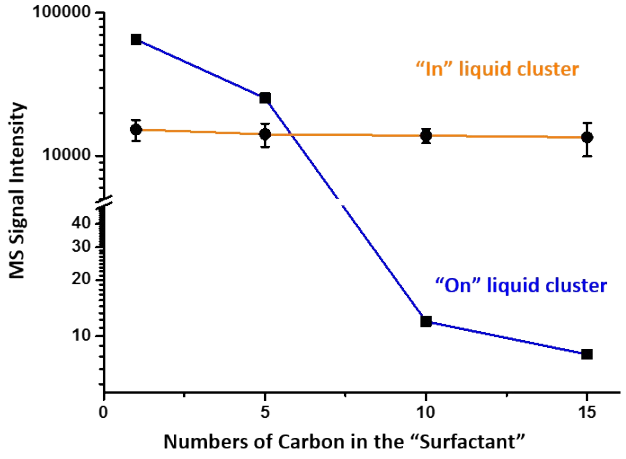


Fig S3 ND-MRESI-MS signal intensities, orange m/z 963 (HomoCIC) and blue m/z 1110 (HeteroCIC), when different "surfactant" of increasing carbon numbers were added in the ESI spray solution (thymine: 0.2 mM, "surfactant": 0.01 mM).

2.4 Optimization of TMA Detection on Fish Meat

The amount of ammonia spiked on fish meat pieces and the concentration of thymine in the ESI spray were optimized. Various concentrations of ammonia were spiked in the Crucian fish meat pieces with 10 ppm TMA added, and the signal intensities of m/z 1110 were recorded accordingly. Based on the data in Fig S4a, 0.1 % v/v ammonia was spiked in later quantitation experiments because it can help to obtain the highest signal intensity. High concentration hamper the heteroCIC formation probably due to complicating the matrix. Subsequently, the thymine concentrations in the ESI solution was optimized. By gradually increasing the thymine concentration from 0.02 mM to 0.2 mM, the obtained signal intensity increased significantly, while further increment of thymine only resulted in minor signal enhancement. To save consumptions of chemicals, 0.2 mM thymine solution was applied in the ESI channel.

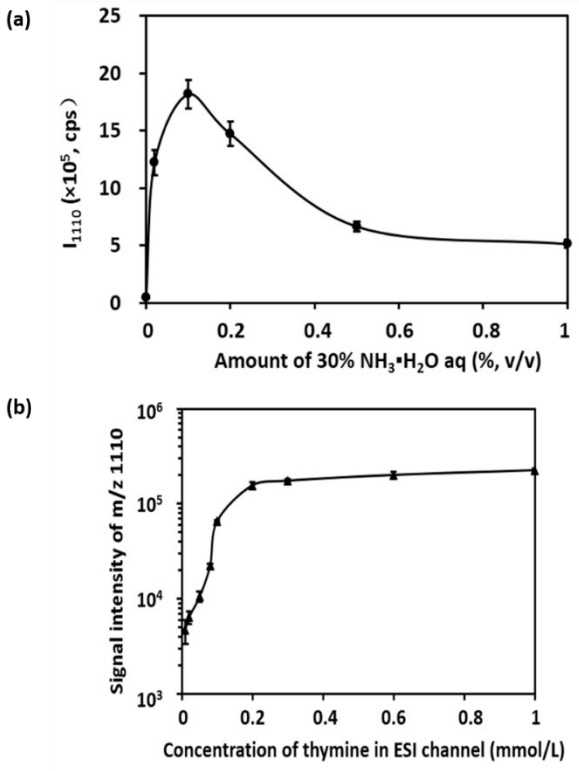


Fig S4 Optimization of (a) ammonia spiked on solid samples and (b) thymine in the ESI spray solution in the quantitation experiments.

2.5 Other Examples of HeteroCICs

Besides TMA, DMA and imidazole can also form HeteroCICs with thymine, assisted by ammonium cation.

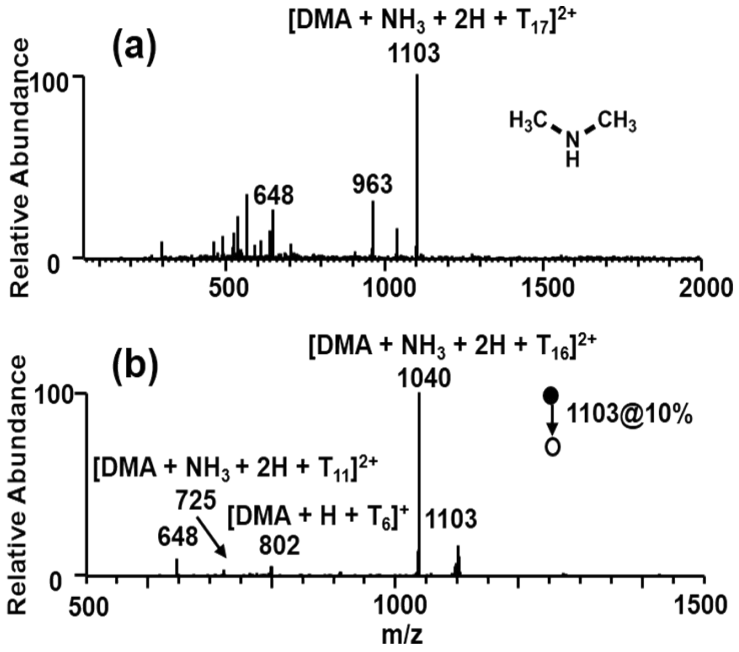


Fig S5 (a) ND-MRESI-MS and (b) MS/MS of the HeteroCIC m/z 1103 formed by DMA, ammonium and thymine.

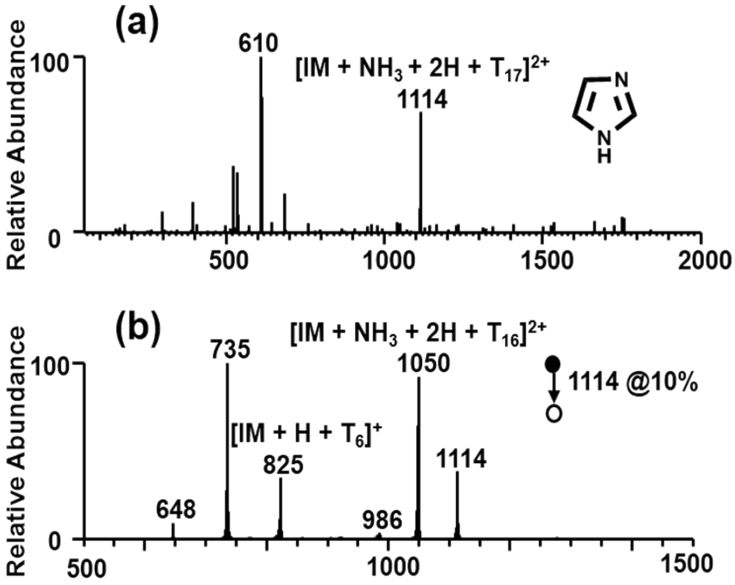


Fig S6 (a) ND-MRESI-MS and (b) MS/MS of the HeteroCIC m/z 1114 formed by imidazole, ammonium and thymine.

2.6 The coordinates of the TMA heteroCIC

O	1.10100000	-3.73250000	-0.36960000
O	-1.42860000	-6.88300000	1.66240000
C	-0.45230000	-6.58820000	0.97450000
N	-0.19570000	-5.24690000	0.61770000
C	0.87480000	-4.94370000	-0.09220000
N	1.71780000	-5.87730000	-0.50370000
C	1.56670000	-7.16160000	-0.22580000
C	0.50250000	-7.60590000	0.52440000
H	-0.81360000	-4.52780000	0.91330000
H	2.51280000	-5.60710000	-1.03900000
H	2.28130000	-7.84820000	-0.57690000
C	0.32850000	-9.06050000	0.87800000
H	0.28280000	-9.16380000	1.96240000
H	-0.59900000	-9.43100000	0.44180000
H	1.16110000	-9.65480000	0.50190000
O	3.37200000	-1.29720000	-2.38180000
O	4.69220000	-5.62240000	-2.35850000
C	4.92220000	-4.44510000	-2.63120000
N	3.96820000	-3.43980000	-2.36130000
C	4.23860000	-2.17870000	-2.64160000
N	5.39160000	-1.82320000	-3.18580000
C	6.35070000	-2.68700000	-3.47660000
C	6.19670000	-4.03200000	-3.22750000
H	3.09030000	-3.68140000	-1.96340000
H	5.55250000	-0.86080000	-3.38860000
H	7.24490000	-2.33420000	-3.90270000
C	7.27690000	-5.03400000	-3.54810000
H	7.57550000	-5.54780000	-2.63410000
H	6.89310000	-5.76410000	-4.26110000
H	8.14930000	-4.54230000	-3.97880000
O	2.91270000	2.50010000	-1.55490000
O	6.52310000	1.41090000	-4.04920000
C	5.88150000	2.25530000	-3.42570000
N	4.68270000	1.91010000	-2.76550000
C	3.98340000	2.83570000	-2.13580000
N	4.38270000	4.09740000	-2.09840000
C	5.49900000	4.51970000	-2.66960000
C	6.31710000	3.65360000	-3.35870000

H	4.36410000	0.96910000	-2.77640000
H	3.82610000	4.76490000	-1.61110000
H	5.76240000	5.53460000	-2.59010000
C	7.60870000	4.10480000	-3.99200000
H	8.44530000	3.63130000	-3.47720000
H	7.62090000	3.81070000	-5.04160000
H	7.71760000	5.18740000	-3.92590000
O	-0.31310000	3.78160000	0.31170000
O	2.74610000	6.91380000	-0.82450000
C	1.66200000	6.62380000	-0.32060000
N	1.22390000	5.28290000	-0.26520000
C	0.05520000	4.98970000	0.27410000
N	-0.73090000	5.93190000	0.77100000
C	-0.41230000	7.21600000	0.77040000
C	0.77940000	7.65230000	0.23830000
H	1.79550000	4.55850000	-0.63330000
H	-1.60740000	5.67030000	1.16520000
H	-1.08470000	7.91040000	1.18420000
C	1.16230000	9.10990000	0.22290000
H	2.09750000	9.24340000	0.76700000
H	1.29690000	9.43640000	-0.80850000
H	0.38990000	9.72070000	0.69030000
O	-3.16350000	1.33650000	1.35090000
O	-3.76040000	5.66310000	2.50890000
C	-4.15250000	4.49730000	2.52820000
N	-3.42400000	3.47830000	1.87940000
C	-3.84370000	2.22840000	1.93090000
N	-4.95470000	1.89730000	2.56970000
C	-5.71050000	2.77740000	3.20640000
C	-5.37610000	4.11260000	3.23750000
H	-2.59410000	3.70080000	1.38120000
H	-5.24210000	0.94250000	2.57300000
H	-6.57820000	2.44610000	3.69860000
C	-6.21720000	5.13510000	3.95860000
H	-5.60970000	5.63430000	4.71400000
H	-6.57920000	5.87430000	3.24340000
H	-7.07140000	4.66440000	4.44550000
O	-2.19740000	-2.48430000	1.28610000
O	-6.06380000	-1.34400000	3.33050000
C	-5.28340000	-2.21510000	2.94980000
N	-4.12160000	-1.87780000	2.22410000
C	-3.25290000	-2.81540000	1.89510000
N	-3.45230000	-4.08550000	2.21020000
C	-4.50920000	-4.50510000	2.88510000

C	-5.48190000	-3.62470000	3.29920000
H	-3.95010000	-0.93090000	1.97600000
H	-2.77500000	-4.76230000	1.93700000
H	-4.59790000	-5.52730000	3.11300000
C	-6.68470000	-4.07560000	4.08610000
H	-6.68240000	-3.58330000	5.05900000
H	-7.59230000	-3.80370000	3.54680000
H	-6.66990000	-5.15540000	4.23480000
O	-1.62130000	-0.36490000	4.02450000
O	-2.30680000	-4.36270000	6.02120000
C	-2.51840000	-3.15180000	5.97670000
N	-1.95260000	-2.36150000	4.95340000
C	-2.12530000	-1.05230000	4.95620000
N	-2.81410000	-0.44610000	5.90980000
C	-3.37290000	-1.09610000	6.91870000
C	-3.27800000	-2.46610000	7.02800000
C	-3.88730000	-3.22450000	8.18270000
H	-1.42380000	-2.79240000	4.23030000
H	-2.91350000	0.54770000	5.87380000
H	-3.89420000	-0.54980000	7.65010000
H	-4.52060000	-2.57420000	8.78620000
H	-4.48990000	-4.04880000	7.80070000
H	-3.09180000	-3.62490000	8.81140000
O	0.94870000	-3.21820000	3.09170000
O	4.73480000	-5.65020000	3.55050000
C	3.54270000	-5.48810000	3.80770000
N	2.82440000	-4.40990000	3.24590000
C	1.56970000	-4.19460000	3.59840000
N	0.95390000	-4.97530000	4.47260000
C	1.54250000	-6.00350000	5.06340000
C	2.84940000	-6.33550000	4.78350000
C	3.54580000	-7.49480000	5.45370000
H	3.26670000	-3.80980000	2.58820000
H	0.00260000	-4.78010000	4.70530000
H	0.99880000	-6.57000000	5.76240000
H	2.85290000	-8.05670000	6.08030000
H	3.95510000	-8.16100000	4.69380000
H	4.35910000	-7.11870000	6.07500000
O	4.35960000	-1.61350000	1.78310000
O	8.19700000	0.75640000	1.48010000
C	7.62580000	-0.30950000	1.70480000
N	6.22290000	-0.41160000	1.58850000
C	5.61540000	-1.54290000	1.89560000
N	6.29110000	-2.60080000	2.31600000

C	7.60640000	-2.60490000	2.46410000
C	8.36060000	-1.49010000	2.17170000
C	9.86130000	-1.46980000	2.33060000
H	5.69370000	0.36940000	1.27460000
H	5.78950000	-3.43660000	2.53240000
H	8.07780000	-3.47830000	2.81090000
H	10.23990000	-2.46310000	2.57210000
H	10.31960000	-1.13360000	1.39990000
H	10.13180000	-0.78190000	3.13180000
O	4.14680000	2.40820000	1.48480000
O	3.53910000	6.19480000	3.87980000
C	4.29690000	5.41910000	3.29970000
N	3.78790000	4.29210000	2.61670000
C	4.61840000	3.41040000	2.09110000
N	5.93130000	3.55270000	2.18450000
C	6.50500000	4.57190000	2.80460000
C	5.75430000	5.56590000	3.39310000
C	6.38620000	6.73350000	4.11450000
H	2.80500000	4.15960000	2.53780000
H	6.52060000	2.85990000	1.77100000
H	7.55450000	4.61590000	2.84620000
H	7.46770000	6.73690000	3.97950000
H	5.97970000	7.66750000	3.72470000
H	6.16430000	6.66530000	5.17950000
O	0.72610000	3.03780000	3.75790000
O	-3.02400000	3.27420000	6.27900000
C	-1.96710000	3.77690000	5.90100000
N	-1.14310000	3.10680000	4.96960000
C	-0.00330000	3.65180000	4.58570000
N	0.39980000	4.82300000	5.05590000
C	-0.28770000	5.51600000	5.94920000
C	-1.49460000	5.06060000	6.43240000
C	-2.28220000	5.82160000	7.47240000
H	-1.42080000	2.22180000	4.60940000
H	1.26800000	5.19800000	4.73380000
H	0.10350000	6.42830000	6.29690000
H	-1.84860000	6.80660000	7.64800000
H	-3.31050000	5.94440000	7.13130000
H	-2.27970000	5.26200000	8.40850000
O	-3.10850000	3.58660000	-2.96340000
O	-7.07290000	5.01050000	-1.33830000
C	-5.98310000	5.24190000	-1.84990000
N	-5.07230000	4.20830000	-2.12870000
C	-3.91380000	4.51550000	-2.67840000

N	-3.62080000	5.77560000	-2.96600000
C	-5.55690000	6.60230000	-2.21550000
H	-5.28160000	3.26770000	-1.89910000
H	-2.71950000	5.97180000	-3.34580000
C	-6.45720000	7.78640000	-1.97100000
H	-7.42060000	7.62730000	-2.45790000
H	-6.60430000	7.88210000	-0.90180000
H	-6.01220000	8.71020000	-2.34540000
N	-4.38000000	6.73460000	-2.73680000
H	-4.05330000	7.64490000	-2.98190000
O	0.40150000	2.72490000	-4.72300000
O	-0.85070000	7.06850000	-4.75000000
C	-0.05800000	6.19090000	-5.08750000
N	-0.25240000	4.85110000	-4.68920000
C	0.59830000	3.91960000	-5.07650000
N	1.64430000	4.20520000	-5.83640000
C	1.90460000	5.42520000	-6.27550000
C	1.09260000	6.48710000	-5.94720000
H	-1.03310000	4.60880000	-4.12230000
H	2.26070000	3.46730000	-6.10160000
H	2.74340000	5.57850000	-6.89120000
C	1.35650000	7.88420000	-6.44860000
H	0.50790000	8.21740000	-7.04650000
H	1.48310000	8.55520000	-5.59890000
H	2.25630000	7.91510000	-7.06300000
O	0.86850000	-1.27240000	-5.68190000
O	4.08180000	1.73540000	-6.69150000
C	3.61700000	0.60460000	-6.81800000
N	2.38580000	0.26450000	-6.21810000
C	1.97440000	-0.98880000	-6.22110000
N	2.68550000	-1.94840000	-6.79500000
C	3.81740000	-1.71730000	-7.43980000
C	4.36020000	-0.45310000	-7.50940000
H	1.83100000	0.96950000	-5.78850000
H	2.34410000	-2.88540000	-6.75890000
H	4.30810000	-2.51970000	-7.90740000
C	5.64270000	-0.15770000	-8.25340000
H	5.42060000	0.43320000	-9.14380000
H	6.31290000	0.41090000	-7.60780000
H	6.14360000	-1.07840000	-8.55620000
O	-1.77500000	-4.22310000	-3.94140000
O	2.10460000	-5.28740000	-6.00020000
C	1.04090000	-5.64500000	-5.49770000
N	0.11460000	-4.69350000	-5.01850000

C	-0.95040000	-5.08950000	-4.34700000
N	-1.17910000	-6.37260000	-4.10950000
C	-0.39950000	-7.34110000	-4.56380000
C	0.74100000	-7.06410000	-5.28480000
H	0.26470000	-3.72490000	-5.18660000
H	-1.98500000	-6.62730000	-3.57850000
H	-0.66330000	-8.34040000	-4.37110000
C	1.64340000	-8.14930000	-5.82420000
H	1.65100000	-8.11270000	-6.91480000
H	2.65720000	-7.99060000	-5.45540000
H	1.30420000	-9.13580000	-5.50720000
O	-4.60290000	-2.99750000	-1.22270000
O	-3.61690000	-7.36760000	-1.79800000
C	-4.37420000	-6.49850000	-1.37120000
N	-4.06280000	-5.13260000	-1.52580000
C	-4.88110000	-4.21440000	-1.04760000
N	-5.99130000	-4.53880000	-0.40220000
C	-6.38550000	-5.78980000	-0.23270000
C	-5.63260000	-6.84160000	-0.70230000
H	-3.23030000	-4.86410000	-1.99950000
H	-6.57010000	-3.80970000	-0.04430000
H	-7.29740000	-5.97710000	0.25660000
C	-6.08400000	-8.27340000	-0.56960000
H	-6.28650000	-8.67930000	-1.56140000
H	-5.29690000	-8.85920000	-0.09530000
H	-6.98970000	-8.33940000	0.03280000
O	-5.28090000	0.94870000	-1.03070000
O	-8.05070000	-2.00440000	0.97520000
C	-7.80880000	-0.85340000	0.61540000
N	-6.59930000	-0.54050000	-0.03910000
C	-6.36670000	0.69580000	-0.43880000
N	-7.24140000	1.66830000	-0.23280000
C	-8.40430000	1.47760000	0.36810000
C	-8.76790000	0.23380000	0.83020000
H	-5.92360000	-1.25340000	-0.19570000
H	-7.02090000	2.58870000	-0.54250000
H	-9.05870000	2.29100000	0.48980000
C	-10.08910000	-0.01240000	1.51160000
H	-10.68350000	-0.70160000	0.91100000
H	-9.91440000	-0.45140000	2.49420000
H	-10.64070000	0.91950000	1.63270000
N	1.28500000	0.26070000	2.02320000
H	0.78810000	-0.09260000	1.20870000
H	2.08310000	-0.33710000	2.21980000

H	1.60780000	1.20760000	1.83800000
H	0.66350000	0.26320000	2.82810000
N	-1.03440000	-0.40320000	-1.87850000
H	-0.57030000	-0.30220000	-0.97180000
C	-2.45720000	-0.71540000	-1.62060000
H	-2.53200000	-1.64780000	-1.06530000
H	-2.90720000	0.07620000	-1.02430000
H	-3.00680000	-0.81640000	-2.55900000
C	-0.88400000	0.87780000	-2.60470000
H	-1.34220000	1.68640000	-2.03450000
H	0.17350000	1.10710000	-2.73660000
H	-1.36160000	0.81550000	-3.58530000
C	-0.36370000	-1.51600000	-2.59140000
H	0.69860000	-1.29350000	-2.71880000
H	-0.46620000	-2.44330000	-2.02160000
H	-0.81260000	-1.66390000	-3.57460000

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