Supporting information

Development of Amide-Based Molecular Cages for the Highly Selective and Sensitive Detection of Nicotine

Mina Bagherifard,[1] Amrit Kaur,[1] Kamal E. S. Nassar,[1] Neelam Tariq,[1] Zois Syrgiannis,[1] and Ioannis Spanopoulos*,[1]

[1]*Department of Chemistry, University of South Florida, Tampa, Florida 33620, United States*

Corresponding author:
spanopoulos@usf.edu

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1. Materials and methods

Reagents

All reagents and chemicals for synthesis were used without further purification. [1,1'-Biphenyl]-4,4'-dicarbaldehyde (97.08%) was purchased from AmBeed. Tris(2-aminoethyl)amine (98%) was purchased from TCI. Ammonium chloride, urea, sodium chloride, potassium chloride, magnesium sulfate, tetrabutylammonium chloride, tetrabutylammonium fluoride, acetone, acetonitrile, methanol, and dimethyl sulfoxide (DMSO) were provided by Fisher Chemical. Sodium chlorite was acquired from Acros Organics. (S)-(-)-Nicotine (99%) was purchased from Alfa Aesar. (1S)-(-)-α-pinene (98%), alpha-Cyano-4-hydroxycinnamic acid, THF, ethyl alcohol (99.5%), and uric acid were acquired from Thermo Scientific. Bovine Albumin serum (BSA) and cholesterol were provided from Gibco and MP Biomedicals, respectively. Calcium chloride, pyridine, pyrrolidine, L-Alanine, L-Argenine, L-Leucine, and glucose were purchased from Sigma Aldrich.

NMR measurements

All NMR measurements were performed using a Bruker Neo 600 MHz NMR spectrometer.

MALDI-TOF mass spectrometry

The mass spectroscopy data was acquired on Bruker UltraFleXtreme MALDI-TOF/TOF. Ultra-pure alpha-Cyano-4-hydroxycinnamic acid matrix was used for sample investigations.

TGA measurements

Thermogravimetric Analysis (TGA) measurements were performed on a TA Instruments Q50 Thermogravimetric Analyzer. About 9 mg sample was placed on an Alumina pan and heated up to 700 °C while the heating ramp was kept 5 °C/minute under an inert atmosphere.

FT-IR Spectroscopy

Fourier-transform infrared (FT-IR) spectroscopy measurements were performed using a PerkinElmer Spectrum Two FT-IR spectrometer, covering a spectral range of 3500-400 cm^{-1}.

UV-Vis Spectroscopy

UV-Vis spectra were recorded on Shimadzu UV-1900i.

PL/TRPL Measurements

PL and TRPL studies were performed using an Edinburgh Instruments FS5 spectrofluorometer equipped with a 150 W xenon lamp and a 300 nm EPLED picosecond pulsed LED.

Quantum Yield Calculation

FS5 Spectrofluorometer equipped with an integrating sphere for photoluminescence quantum yield (PLQY) determination was utilized to measure quantum yield.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) measurements were performed using a high-resolution thermal field emission Hitachi SU-70 microscope at an accelerating voltage of 20 kV. Two different samples were prepared: one without nicotine and one with nicotine. For the sample without nicotine, 200 μL of a 1 mM **BiP-Am** solution was mixed with 800 μL of deionized water. For the sample with nicotine, 50 μL of a nicotine stock solution (0.1 M) was added to the same **BiP-Am** and deionized water mixture.

UV-Vis and Fluorescence sample preparation

For the UV-Vis and fluorescence titrations, a 1 mM stock solution of **BiP-Am** was prepared in DMSO. The nicotine stock solution (0.1 M) was prepared by dissolving 16 μL of S-nicotine in 984 mL of distilled water and was further serially diluted to obtain the desired concentrations. A 10 μM concentration of **BiP-Am** (30 μL of 1 mM BiP-Am stock solution in total 3 mL aqueous sample) was used for each titration. For testing interfering analytes (Na^+, K^+, Ca^{2+}, Mg^{2+}, NH_4^+, Cl^-, F^-, alanine, arginine, leucine, bovine serum albumin (BSA), glucose, cholesterol, urea, uric acid, and nicotine), standard solutions were prepared with a 0.1 M stock solution of each interferes and diluted to appropriate concentrations ranging from 0 to 1.0 mM for spectral recording. In the titration experiments, 3 mL of a **BiP-Am** solution (30 μL of probe in 2970 μL of distilled water) was placed in a quartz cuvette (path length 1 cm), and spectra were recorded after the addition of the appropriate analyte.

Real-world sample preparation

To determine the amount of nicotine in commercially available cigarettes, 250 mg of tobacco leaves were measured and ground to a powder. Subsequently, 5 mL of deionized (DI) water was added to extract the nicotine. The mixture was sonicated for 3 hours, then centrifuged and filtered to obtain a clear nicotine solution. The solution was diluted 100-fold with DI water. Urine samples were also diluted 100-fold and sonicated for 1 hour. The prepared solutions were then filtered and used for further analysis. Standard nicotine solutions were spiked into the cigarette and urine samples and quantified using a calibration curve.

The human urine samples were de-identified urine samples from persons 18-50 years of age. Corresponding IRB protocol # STUDY007287 was evaluated by the University IRB committee, concluding that: “The IRB determined that the proposed activity does not constitute research involving human subjects as defined by DHHS and FDA regulations.” Therefore, no written consent was required.

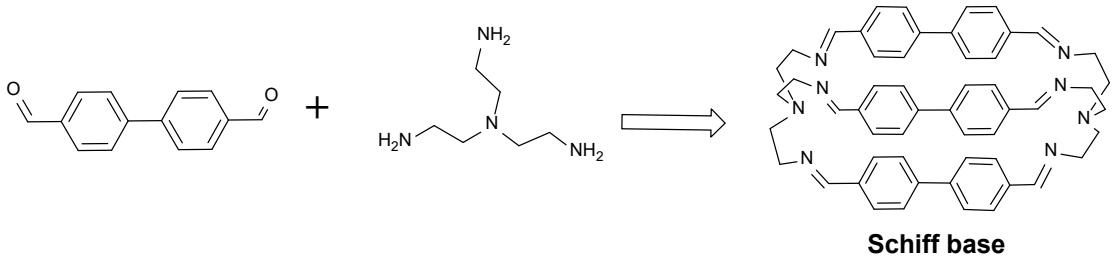
2. Overview of reported nicotine sensors

Table S1. Comparison table of previously reported Nicotine sensors in terms of detection methods and limit of detection (LOD).

No.	Reference	Material Type	Response Techniques	LOD
1.	Current work	Amide-base cages (BiP-Am)	"turn-off" Fluorescent sensors	0.4 nM
2.	*ACS Appl. Bio Mater.* **2024**, *7*, 4, 2346–2353[1]	Zinc based metalloporphyrin	"turn-on" Fluorescent sensors	7.2 nM
3.	*Anal. Methods,* **2023**, *15,* 6377-6384[2]	Gold Nanoparticle AuNPs	Colorimetric sensor	2480 nM
4.	*Inorg. Chem.* **2023**, *62,* 20458−20466[3]	Eu(bpy)@HOF	"turn-off" Fluorescent sensors	591 nM
5.	*J. Mater. Chem. A,* **2023**,*11*, 4739-4750[4]	Tb@HOF	"turn-on" Fluorescent sensors	42 nM
6.	*ACS Appl. Nano Mater.* **2022**, *5*, 16753–16759[5]	Co-based MO	Electrochemical and Fluorescent sensor	250 nM
7.	*Anal. Methods*, **2022**, *14*, 1579-1584[6]	gold screen-printed electrode	electrochemical sensor	39450 nM
8.	*Mater. Adv.*, **2021**, *2*, 3336-3345[7]	graphene/poly(3,4-ethylenedioxythiophene) nanocomposite	electrochemical sensor	47 nM
9.	*Anal. Chem.* **2020**, *92*, 11438–11443[8]	gold nanoclusters (AuNCs)	Electrochemiluminescence	0.0007 nM
10.	*Anal. Methods*, **2020**, *12*, 193-199[9]	Alizarin Red S (ARS) dye	Colorimetric	1080 nM
11.	*Chem. Commun*., **2019**, *55*, 12679-12682[10]	o-carborane derivatives	"turn-off" Fluorescent sensors	18.5 nM

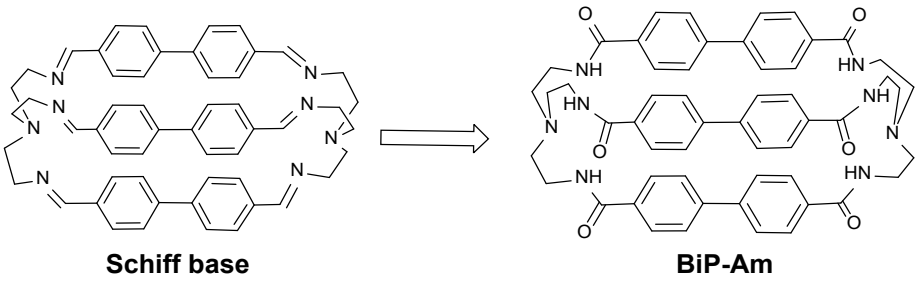
3. Synthesis

BiP-Am cage synthesis:



Scheme S1. The first step of synthesizing the BiP-Am cage is Schiff base formation.

To synthesize the **BiP-Am** cage, Schiff base formation[11] and Pinnick oxidation techniques were employed. In the first step, 1 g of [1,1'-Biphenyl]-4,4'-dicarbaldehyde was dissolved in 150 mL of dry acetonitrile under inert conditions. The temperature was maintained at 0 °C, and a solution of 470 µL Tris(2-aminoethyl)amine in 125 mL of dry acetonitrile was added dropwise over two hours under vigorous stirring and inert conditions. The reaction temperature was kept below 10 °C for 8 hours, followed by room temperature for 7 days. The products were then separated from the solution by vacuum filtration (1102 mg, 85%).



Scheme S2. The second step of synthesizing BiP-Am cage; Pinnick oxidation.

In the second step, 0.6 mmol of the synthesized Schiff base was suspended in 100 mL of dry THF under inert conditions. To this suspension, 20.1 mmol of sodium chlorite, 7.4 mmol of ammonium chloride, and 36.8 mmol of (1S)-(-)-α-pinene were added. The solution was then kept under reflux for 48 hours to obtain the white **BiP-Am** product. The synthesized **BiP-Am** was filtered and washed sequentially with methanol, water, and acetone, and then stored in a desiccator for drying (463 mg, 73%).

^{1}H NMR (600 MHz, DMSO-d_6): δ_H ppm = 2.65 - 2.76 (m, 12 H) 3.48 - 3.57 (m, 12 H) 7.16 (d, J=8.17 Hz, 12 H) 7.44 (d, J=8.17 Hz, 12 H) 7.73 (br t, J=4.81 Hz, 6 H)

^{13}C NMR (151 MHz, DMSO-d_6): δ_C ppm = 35.86, 50.56, 126.05, 127.22, 133.00, 140.66, 165.91.

$[M+H]^+$ ion = m/z = 911.421

4. Characterization

NMR measurements

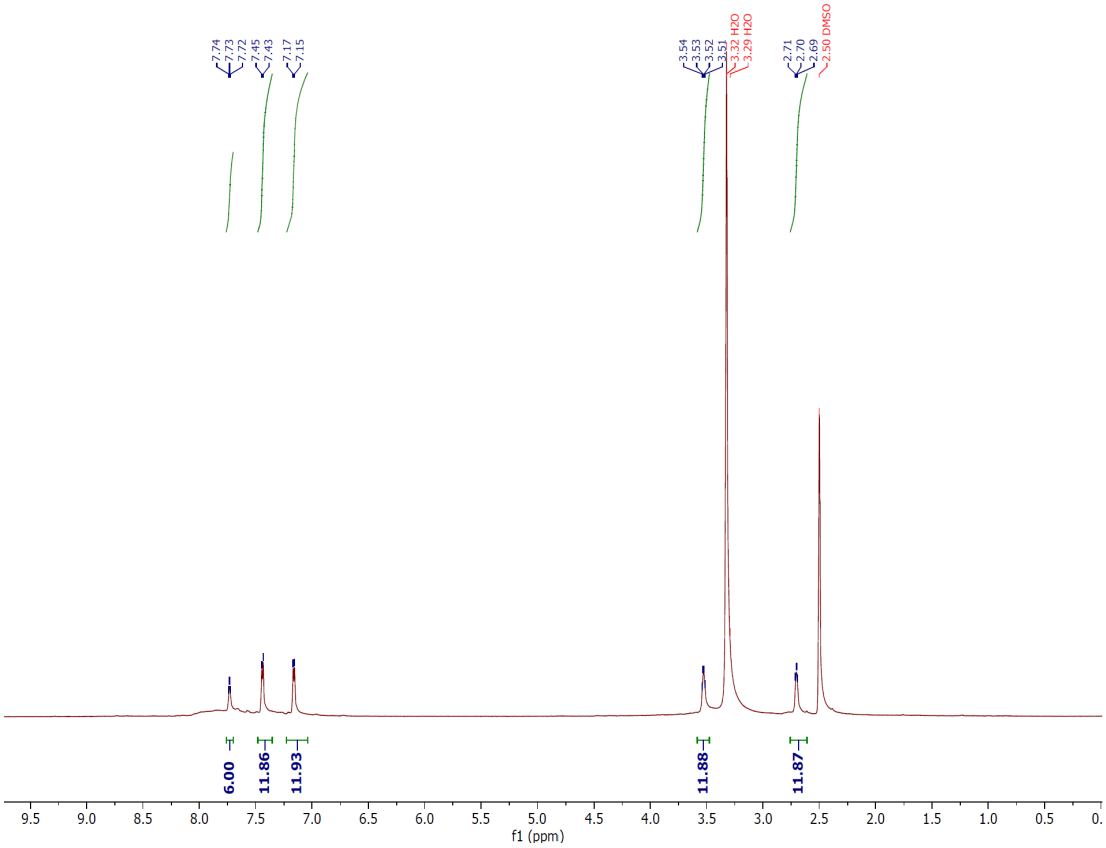


Figure S1. ^{1}H NMR spectrum of the **BiP-Am** cage.

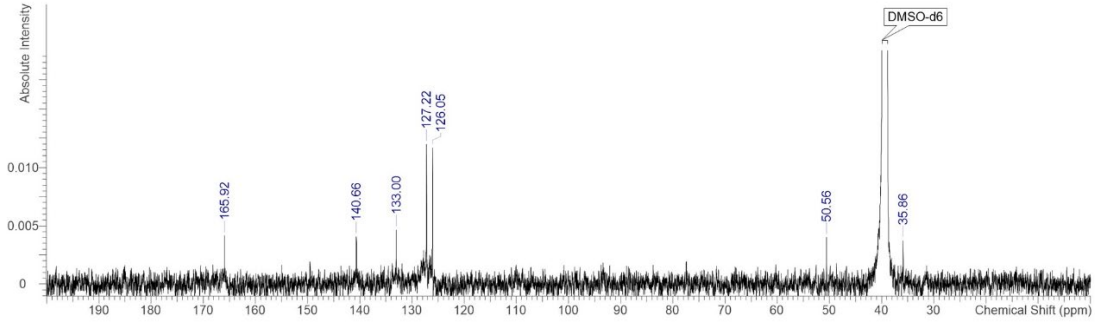


Figure S2. ^{13}C NMR spectrum of the **BiP-Am** cage.

MALDI-TOF mass spectrometry

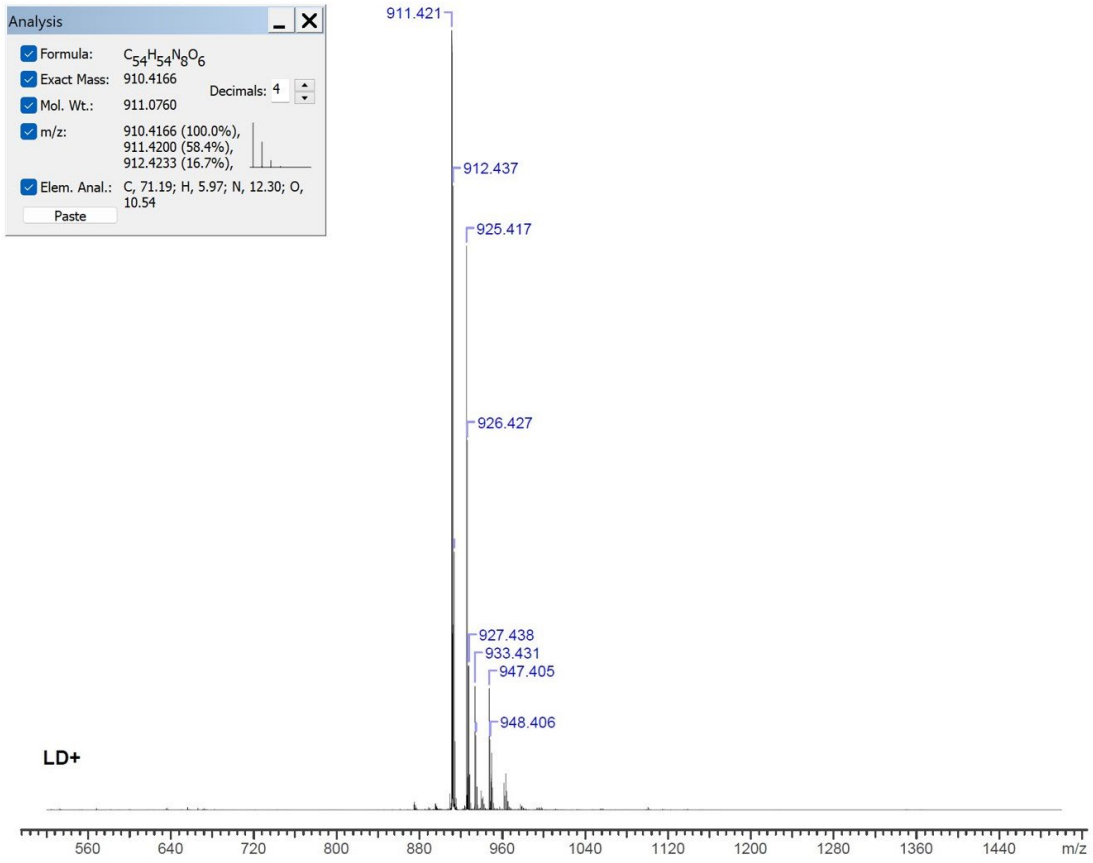


Figure S3. MALDI-TOF mass spectrometry data for the **BiP-Am** cage showed a $[M+H]^+$ ion at m/z = 911.421.

FT-IR spectroscopy

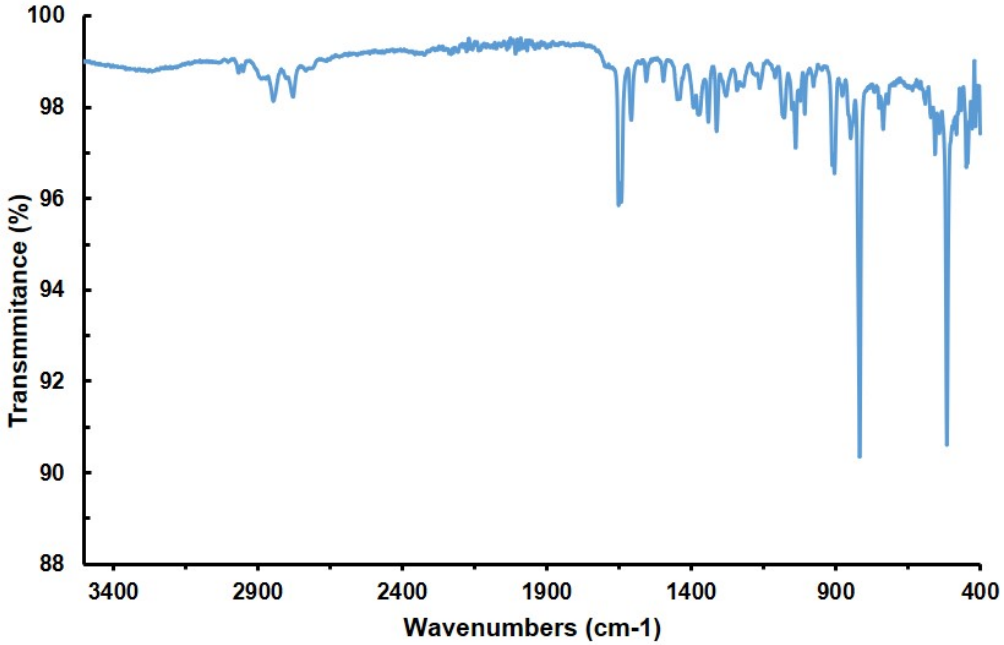


Figure S4. The FT-IR spectrum of intermediate **Schiff base**; ν_{max}/cm^{-1} 1652 corresponds to the C=N double bonds.

Thermogravimetric analysis

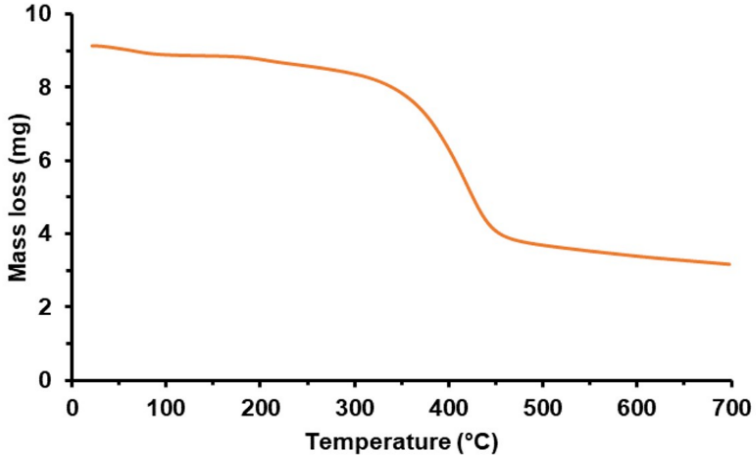


Figure S5. TGA curve for the **BiP-Am** cage.

5. Properties

Solvatochromism

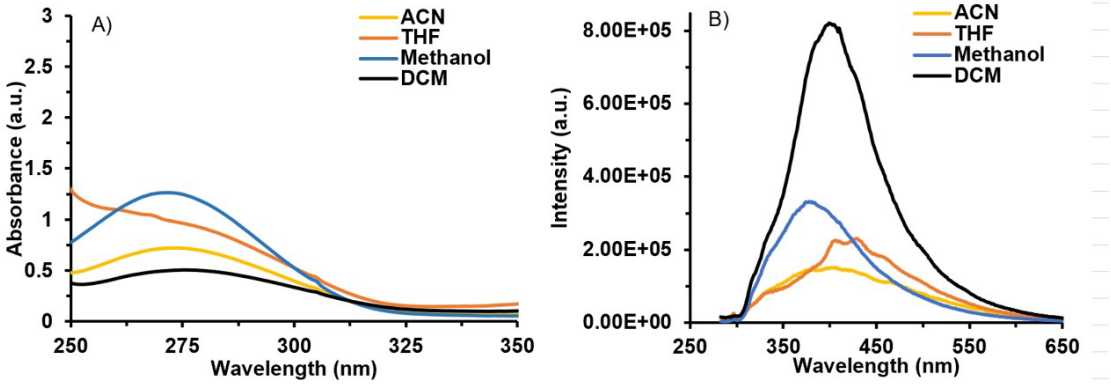


Figure S6. A) absorption, and B) emission spectra of the **BiP-Am** probe under excitation at 273 nm in various solvents (solvent:DMSO (9.9:0.1, v:v, 10 μM))

Absorption/emission spectra of various water fractions

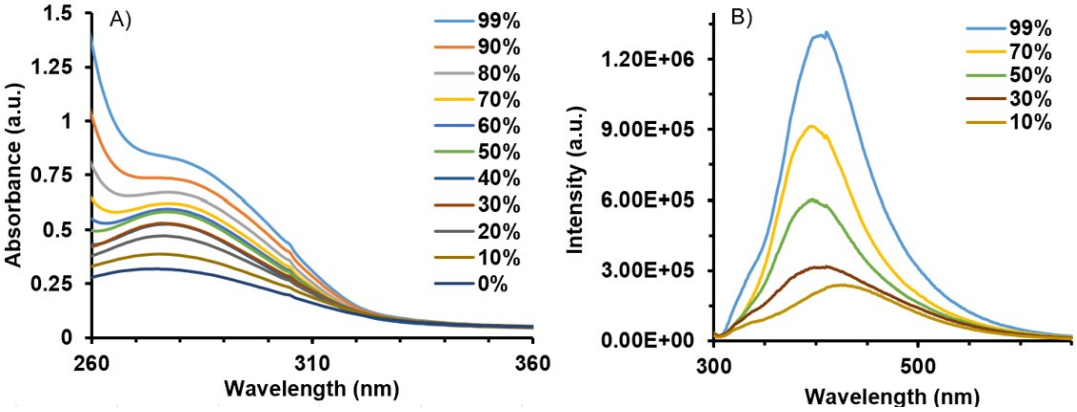


Figure S7. A) absorption, and B) emission spectra of the **BiP-Am** probe under excitation at 273 nm upon varying water fraction in the DMSO from 0-99% by volume; H_2O:DMSO (9.9:0.1, v:v, 10 μM) shows the most enhanced peak in both absorption and emission spectra.

UV-Vis spectra of nicotine titration

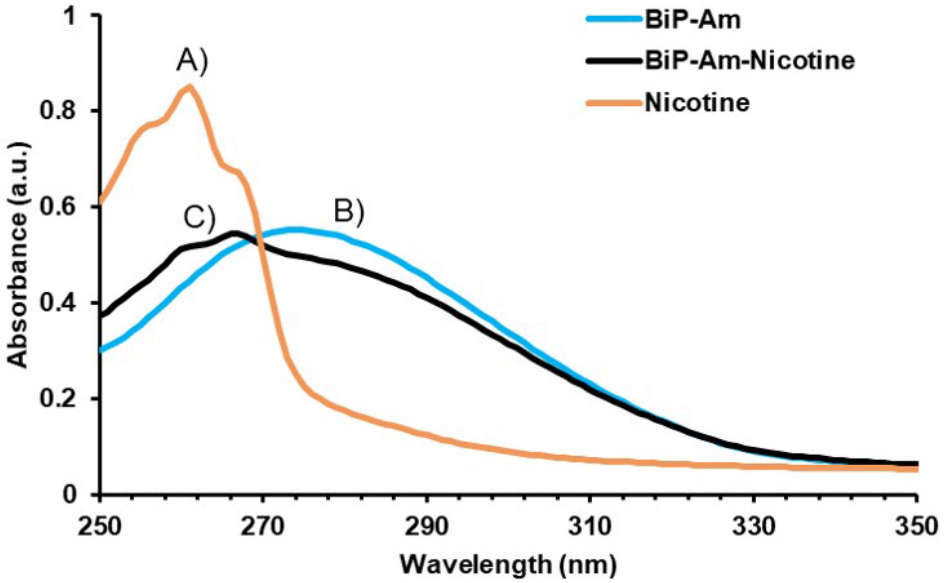


Figure S8. A) The UV-Vis spectrum of nicotine (0.1 M), B) **BiP-Am** probe in H_2O:DMSO (9.9:0.1, v:v, 10 μM), and C) the change in absorbance after the addition of nicotine (10 μL of 1 mM solution) to the **BiP-Am** probe in H_2O:DMSO (9.9:0.1, v:v, 10 μM).

Selectivity studies

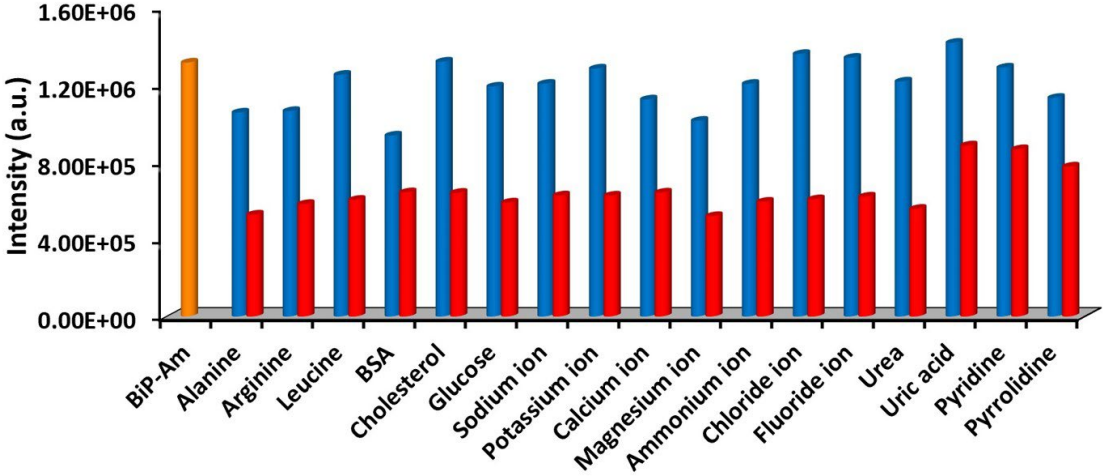


Figure S9. Selectivity assessment of the BiP-Am probe in H_2O:DMSO (9.9:0.1, v/v, 10 μM). The orange column represents the emission intensity of pure BiP-Am, while the blue columns depict the changes in emission upon the addition of various interfering analytes. The red columns represent the emission intensity in the presence of both the interferent and nicotine. All emission spectra were recorded under excitation at 273 nm.

Stern-Volmer diagram

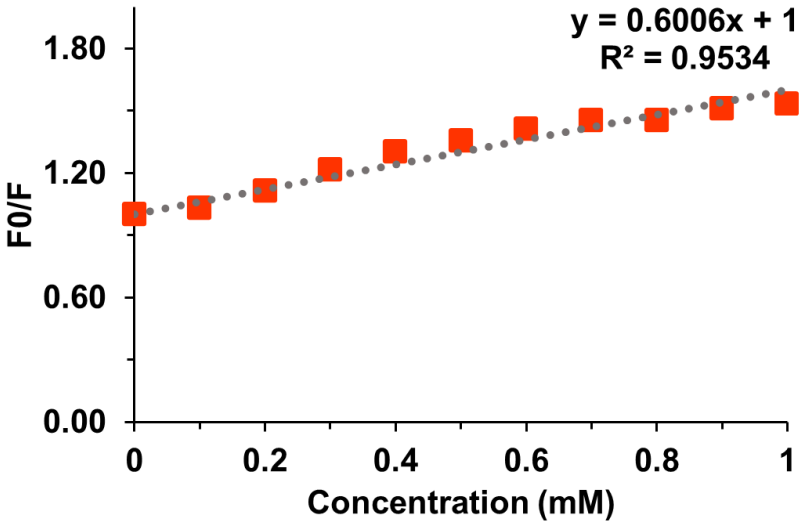


Figure S10. Stern-Volmer diagram of **BiP-Am** in H_2O:DMSO (9.9:0.1, v:v, 10 μM) upon addition of 0.1 M Nicotine to the solution.

Time-resolved photoluminescence studies

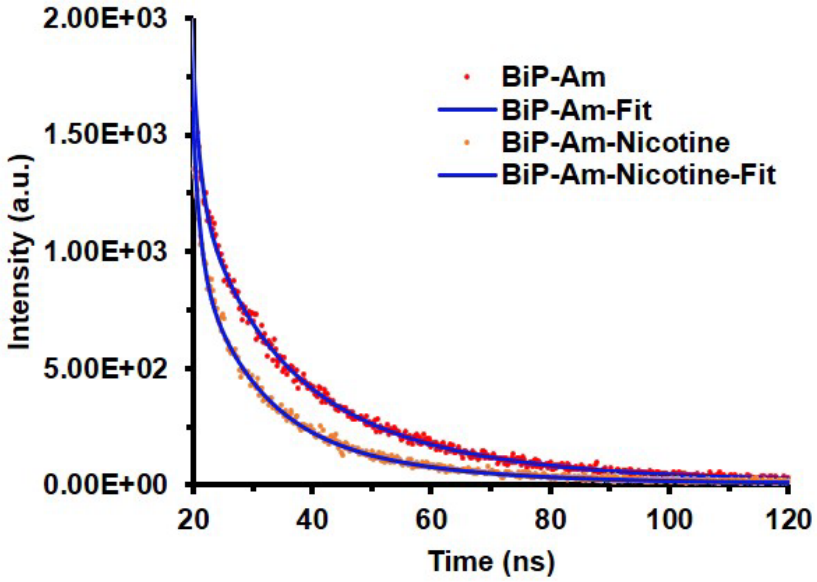


Figure S11. Time-resolved photoluminescence decay of the **BiP-Am** before and after the addition of nicotine to the sample in H_2O:DMSO (9.9:0.1, v:v, 10 μM).

^{1}H NMR nicotine titration

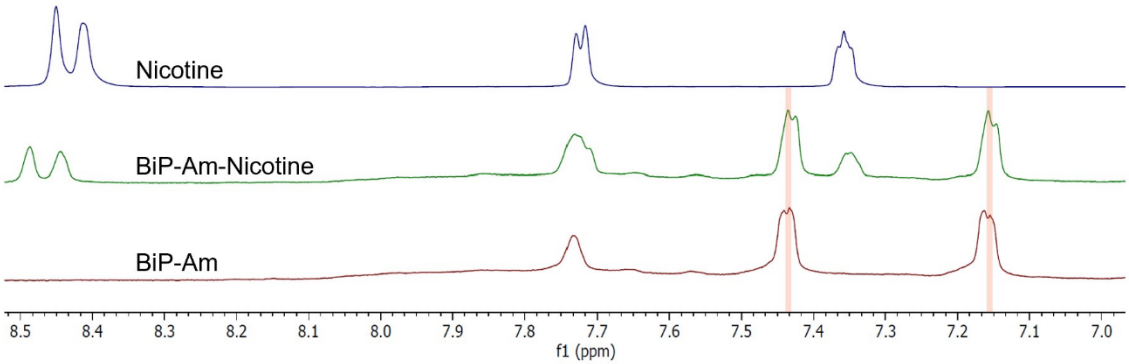


Figure S12. ^{1}H NMR spectra of **BiP-Am**, **BiP-Am-Nicotine** and **Nicotine** in DMSO-d_6:D_2O (9:1, v:v).

DLS measurements

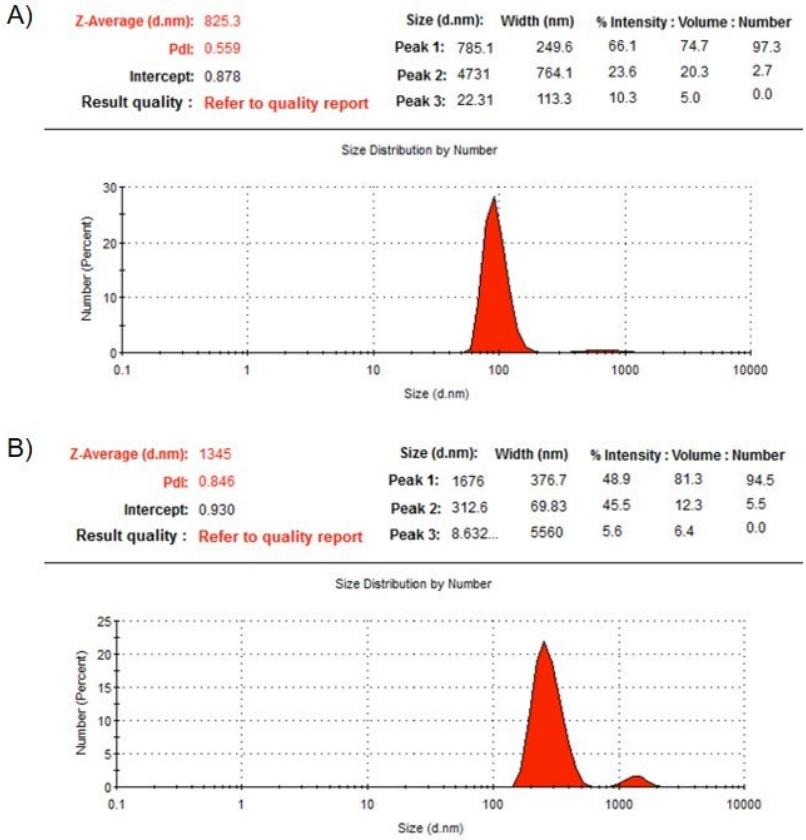


Figure S13. DLS data of A) **BiP-Am** and B) after the addition of nicotine to **BiP-Am** probe in H_2O:DMSO (9.9:0.1, v:v, 10 μM).

Emission spectra under different excitation wavelengths

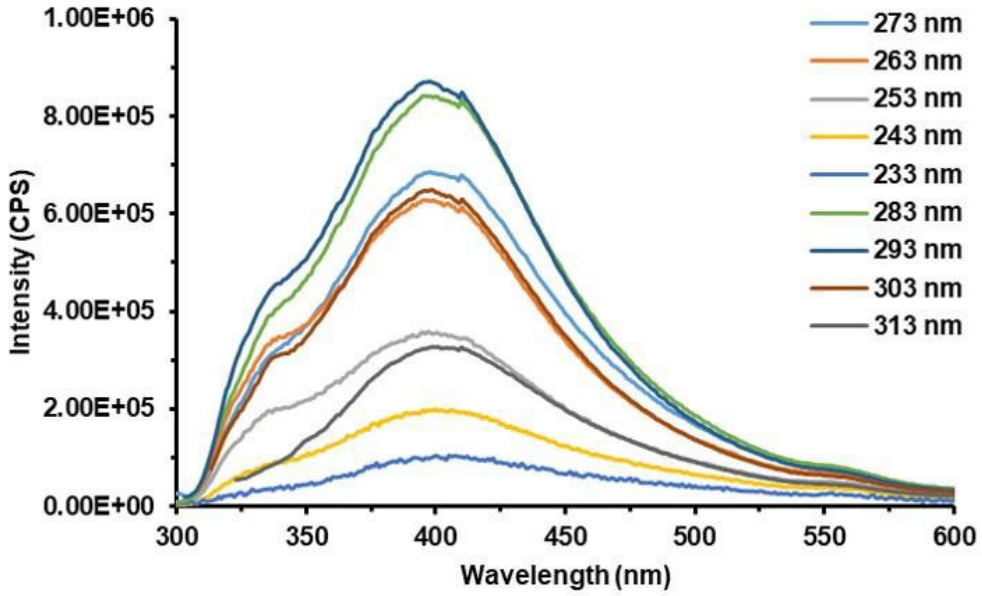


Figure S14. The emission spectra of probe **BiP-Am-Nicotine** in H_2O:DMSO (9.9:0.1, v:v, 10 μM) under various excitation wavelengths (263-313 nm).[12]

Absorption and emission spectra of Nicotine and BiP-Am

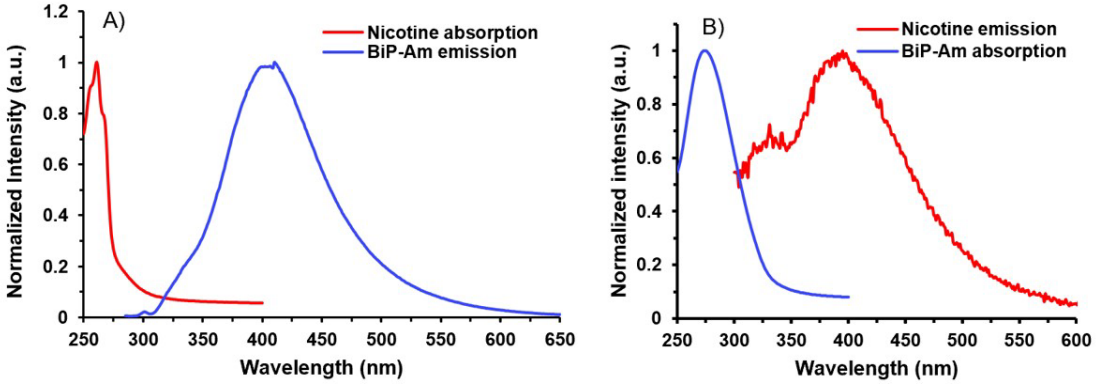


Figure S15. Normalized absorption and emission spectra of Nicotine and **BiP-Am** in H_2O:DMSO (9.9:0.1, v:v, 10μM) under excitation at 263 nm and 273 nm, respectively.

BiP-Am emission in different matrices before/after nicotine addition

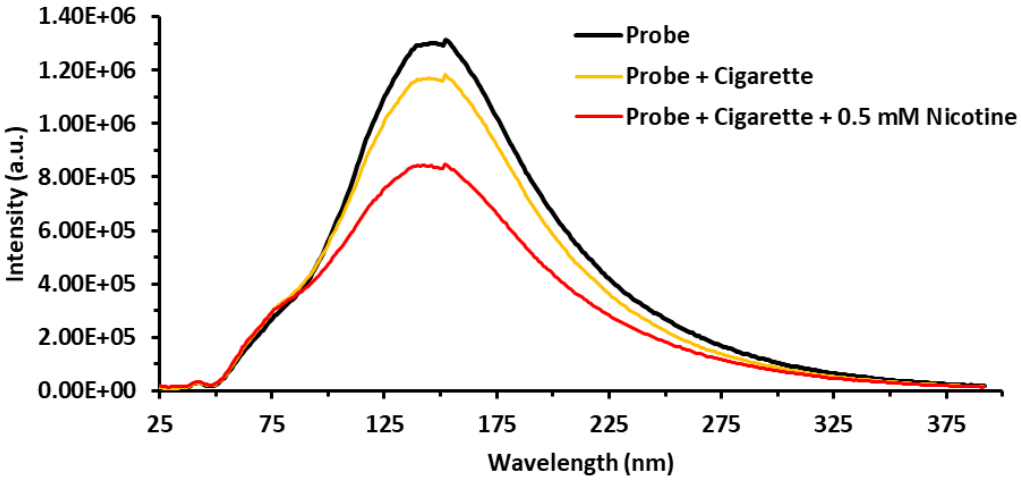


Figure S16. The emission spectra of **BiP-Am** in H_2O:DMSO (9.9:0.1, v:v, 10μM) under excitation at 273 nm (black); after the addition of 50 μL of prepared cigarette solution (orange); and after the addition of 0.5 mM Nicotine (red).

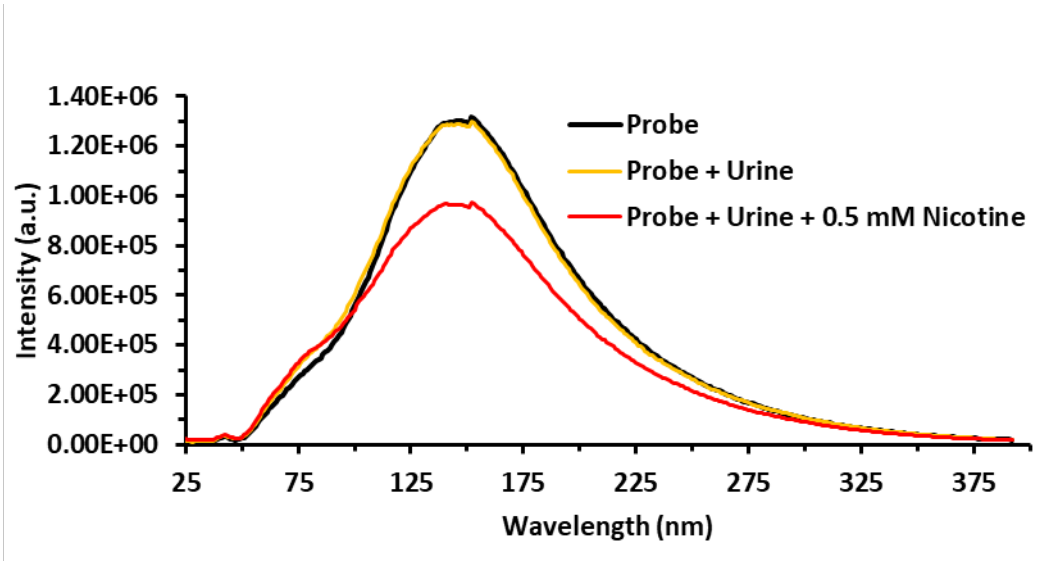


Figure S17. The emission spectra of **BiP-Am** in H_2O:DMSO (9.9:0.1, v:v, 10μM) under excitation at 273 nm (black); after the addition of 50 μL of prepared urine solution (orange); and after the addition of 0.5 mM Nicotine (red).

Table S2. DFT and TD-DFT calculated data for ground and excited states.

Samples	Basis Set	Solvent	HOMO-Gr (eV)	HOMO-Ex (eV)	LUMO-Gr (eV)	LUMO-Ex (eV)
Nicotine	6-31G(d)	Water	-5.508	-4.639	-0.756	-1.171
BiP-Am	6-31G(d)	Water	-5.966	-5.029	-1.567	-2.330

BiP-Am coordinates			
N	-1.90331	3.92392	-2.25085
C	-3.00278	2.99929	-1.87026
H	-3.72547	3.46824	-1.19181
H	-3.56864	2.72949	-2.77183
C	-2.30269	4.78756	-3.38793
H	-2.88833	5.64824	-3.04146
H	-2.94708	4.23042	-4.08022
C	-1.45903	4.76964	-1.11693
H	-0.75118	5.52085	-1.48794
H	-2.30531	5.33612	-0.70479
C	-0.73961	4.02823	0.02157
H	0.01329	3.36227	-0.40564
H	-1.44694	3.44683	0.61649
C	-1.08857	5.2658	-4.21742
H	-0.38317	5.83033	-3.60325
H	-1.44591	5.9231	-5.01721
C	-2.50123	1.65441	-1.3014
H	-2.31548	1.70852	-0.226
H	-3.25311	0.87251	-1.4581
N	-1.26895	1.24489	-1.94483
H	-0.85463	1.91633	-2.58401
N	-0.40111	4.15432	-4.86461
H	-0.94982	3.32685	-5.0684
N	-0.11382	4.95846	0.95032
H	-0.64025	5.77046	1.25173
C	-0.68392	0.02215	-1.72657
C	0.91325	4.00046	-5.21448
C	1.15588	4.92996	1.46559
O	-1.15088	-0.83679	-0.98962
O	1.29411	2.91578	-5.65044
O	1.58244	5.8883	2.10237
C	0.559	-0.23043	-2.4984
C	0.76482	-1.50097	-3.04097
C	1.4939	0.78224	-2.72475
C	1.86623	-1.73628	-3.87131
H	0.0452	-2.29621	-2.85786
C	2.5971	0.53524	-3.54599
H	1.37435	1.76743	-2.28331
C	2.78626	-0.71438	-4.16149

Nicotine coordinates			
C	-1.0397	-0.2551	0.7525
C	-0.3708	0.9696	0.7066
C	0.6631	1.152	-0.2112
N	1.01	0.144	-1.0526
C	0.3753	-1.0579	-1.029
C	-0.6692	-1.2977	-0.1185
C	-1.3643	-2.6556	-0.1305
C	-2.5902	-2.8171	0.7689
C	-2.825	-4.2998	0.6057
C	-1.4116	-4.82	0.8051
N	-0.5043	-3.8348	0.1645
C	0.6442	-3.563	1.0517
H	-1.8397	-0.3737	1.4692
H	-0.6526	1.7715	1.3767
H	1.1888	2.0963	-0.2572
H	0.684	-1.8308	-1.7214
H	-1.7361	-2.7841	-1.1747
H	-3.4568	-2.1881	0.4723
H	-2.3234	-2.6099	1.8307
H	-3.1467	-4.4991	-0.4426
H	-3.5745	-4.741	1.2966
H	-1.2736	-5.8464	0.4053
H	-1.2444	-4.8554	1.9083
H	1.1612	-4.5133	1.3053
H	0.3188	-3.0635	1.9908
H	1.394	-2.928	0.541

H	3.30147	1.34312	-3.73016
C	1.9	5.0935	-5.04855
C	3.19196	4.74017	-4.6281
C	1.62165	6.41914	-5.38271
C	4.20919	5.69938	-4.49287
H	3.4104	3.69167	-4.435
C	2.61084	7.39102	-5.2362
H	0.65463	6.70429	-5.78366
C	3.88743	7.03418	-4.78953
H	2.39749	8.4277	-5.48657
H	4.63997	7.81439	-4.68489
C	2.02437	3.756	1.23645
C	1.59194	2.46202	1.5374
C	3.32107	3.96886	0.76777
C	2.44917	1.37671	1.33165
H	0.60013	2.2891	1.94681
C	4.17417	2.88011	0.57015
H	3.67018	4.97903	0.5651
C	3.75525	1.56556	0.84743
H	2.09604	0.377	1.58183
H	5.18026	3.06837	0.20159
C	3.88506	-0.92143	-5.12279
C	4.10011	0.00091	-6.16685
C	4.72637	-2.04604	-5.04908
C	5.12702	-0.1823	-7.09614
H	3.44543	0.86645	-6.27501
C	5.75221	-2.24104	-5.98009
H	4.58851	-2.78184	-4.25921
C	5.95574	-1.29781	-6.98783
H	5.27102	0.53664	-7.89812
H	6.38515	-3.1215	-5.91511
C	5.57249	5.30526	-4.08736
C	5.78882	4.44346	-2.99596
C	6.69872	5.76737	-4.79288
C	7.07969	4.04408	-2.63126
H	4.9454	4.08265	-2.41015
C	7.99162	5.3751	-4.43231
H	6.5775	6.43118	-5.6472
C	8.17821	4.49464	-3.36615
H	7.21916	3.39571	-1.77108
H	8.84829	5.7502	-4.98793
C	4.67139	0.42574	0.66478
C	4.26788	-0.73388	-0.02392
C	5.97855	0.46749	1.18611
C	5.14293	-1.80985	-0.20172
H	3.26416	-0.80522	-0.44031
C	6.86109	-0.59911	1.00767

H	6.31421	1.33382	1.75377
C	6.44067	-1.72632	0.30026
H	4.81632	-2.69644	-0.73901
H	7.86501	-0.55089	1.42067
C	7.38675	-2.83119	0.0614
C	9.55827	4.10078	-3.0101
C	7.05259	-1.47508	-7.95679
O	7.22096	-3.91456	0.60789
O	6.85502	-2.08223	-9.00211
O	10.4087	4.96525	-2.82379
N	8.44739	-2.6118	-0.78245
H	9.09546	-3.38966	-0.82805
N	8.28302	-0.93928	-7.66315
H	8.97608	-1.0858	-8.38745
N	9.89182	2.77319	-2.91702
H	10.84364	2.6294	-2.59938
C	8.67952	-1.44269	-1.61803
H	7.75439	-0.86534	-1.70177
H	9.4265	-0.82505	-1.10904
C	9.15497	-1.83013	-3.02903
H	10.1805	-2.2195	-2.98821
H	8.52011	-2.66311	-3.36015
C	8.67312	-0.25385	-6.44094
H	7.81298	0.30377	-6.05761
H	9.44416	0.47578	-6.70975
C	9.20836	-1.23537	-5.37821
H	8.65505	-2.17627	-5.48252
H	10.25756	-1.4823	-5.58692
C	9.07099	1.61501	-3.23259
H	8.51687	1.36467	-2.32265
H	8.34983	1.88612	-4.01074
C	9.91333	0.41535	-3.70287
H	10.49326	0.72622	-4.5815
H	10.66697	0.16503	-2.94506
N	9.04205	-0.73089	-4.00455
H	1.97255	-2.71722	-4.3309

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