frontiers

Supplementary Material

Exploring the influence of free radicals on photolytic removal of nadolol from water: Mechanism of degradation and toxicity of intermediates

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Materials and methods

Chemicals and Solutions

The chemicals used for UFLC were H_3PO_4 (85%, Sigma–Aldrich) and acetonitrile (ACN, 99.9%, Sigma–Aldrich). Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) from PAA Laboratories GmbH (Pashing, Austria), EDTA, trypsin from Serva (Heidelberg, Germany) tris(hydroxymethyl)amino methane (TRIS) from Sigma–Aldrich and trichloroacetic acid (TCA) were used for toxicity analysis.

The stock solution of 0.05 mmol/L of NAD was made using ultrapure water (UPW), protected from light, and stored at a temperature of 25 ± 1 °C.

Degradation procedures

Direct and indirect photolysis under different irradiation (under simulated solar (SS), UV-LED, and UV radiations) were carried out in a cell made of Pyrex glass (total volume of *ca.* 40 mL, liquid layer thickness 35 mm), with a plain window on which the light beam was focused. The cell had a magnetic stirring bar and a circulating water jacket. The pH change for all examined processes is given in Table S1. Before irradiation, the solution was set at 25 ± 0.2 °C in a stream of O_2. The mixture was stirred at a constant rate during irradiation under continuous gas flow. The flow of O_2 was 3.0 L/min in all experiments of degradation.

Energy fluxes measurement. The UV energy fluxes were measured using a Delta Ohm HD 2102.2 (Padova, Italy). The radiometer was fitted with the LP 471 UVA sensor (spectral range 315–400 nm), and in a case of Vis energy radiometer was fitted with the LP 471 RAD (spectral range 400–1050 nm).

Analytical procedures

UFLC–PDA analysis. Aliquot sampling caused a maximum volume variation of *ca.* 10% in the reaction mixture. The gradient method of analysis was utilized in *UFLC*–PDA. The mobile phase (flow rate 0.7 cm^3/min) was a mixture of ACN and water (0 min 15% ACN, which increased to 25% ACN for 6 min, after which 25% ACN was constant for the next 2 min; post time 1 min), the water is acidified with 0.1% H_3PO_4. The retention time for NAD was 5.8 ± 0.1 min. The reproducibility of repeated runs was around 3−10%.

Cell growth activity. HT-29 and MRC-5 were grown in DMEM medium with 4.5% glucose, supplemented with 10% heat-inactivated FCS, 100 IU/mL of penicillin, and 100 mg/L of streptomycin. They were cultured in 25 cm^2 flasks (Corning, New York, USA) at 37 °C in the atmosphere of 5% CO_2 and high humidity, sub-cultured twice a week, and a single cell suspension was obtained using 0.1% trypsin with 0.04% EDTA. The concentration of NAD solution used for photolysis was 0.05 mmol/L. A volume of 20 μL was added to 180 μL of the cell culture medium so that the final dilution was 10. The same volume of UPW (20 μL) was added to the control wells. All samples were filtered through the membrane filters of 0.22 μm Millipore (Millex-GV, 0.22 μm).

Table S1. The pH change during photolysis of 0.05 mmol/L NAD in the absence (direct photolysis) and presence (indirect photolysis) of 3 mmol/L H_2O_2 under different irradiation types

Photodegradation process	**Time**	**pH**
Direct photolysis NAD/SS	0	8.2
	15	8.7
	30	8.7
	60	9.0
	120	9.0
	180	9.1
Direct photolysis NAD/UV-LED	0	8.1
	15	8.4
	30	8.5
	60	8.6
	120	8.8
	180	8.8
Direct photolysis NAD/UV	0	8.2
	15	8.8
	30	8.5
	60	9.0
	120	9.7
	180	9.2
Indirect photolysis NAD/SS	0	7.9
	15	8.1
	30	8.2
	60	8.3
	120	8.0
	180	7.1
Indirect photolysis NAD/UV-LED	0	7.7
	15	7.0
	30	6.7
	60	6.3
	120	5.8
	180	5.6
Indirect photolysis NAD/UV	0	7.7
	15	7.6
	30	7.3
	60	7.0
	120	6.9
	180	6.8

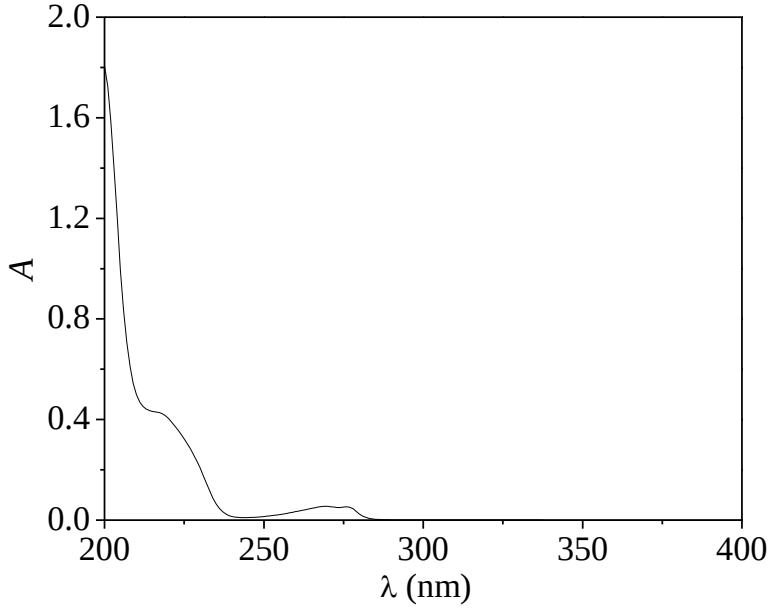


Figure S1. Absorption spectra of NAD (0.05 mmol/L).

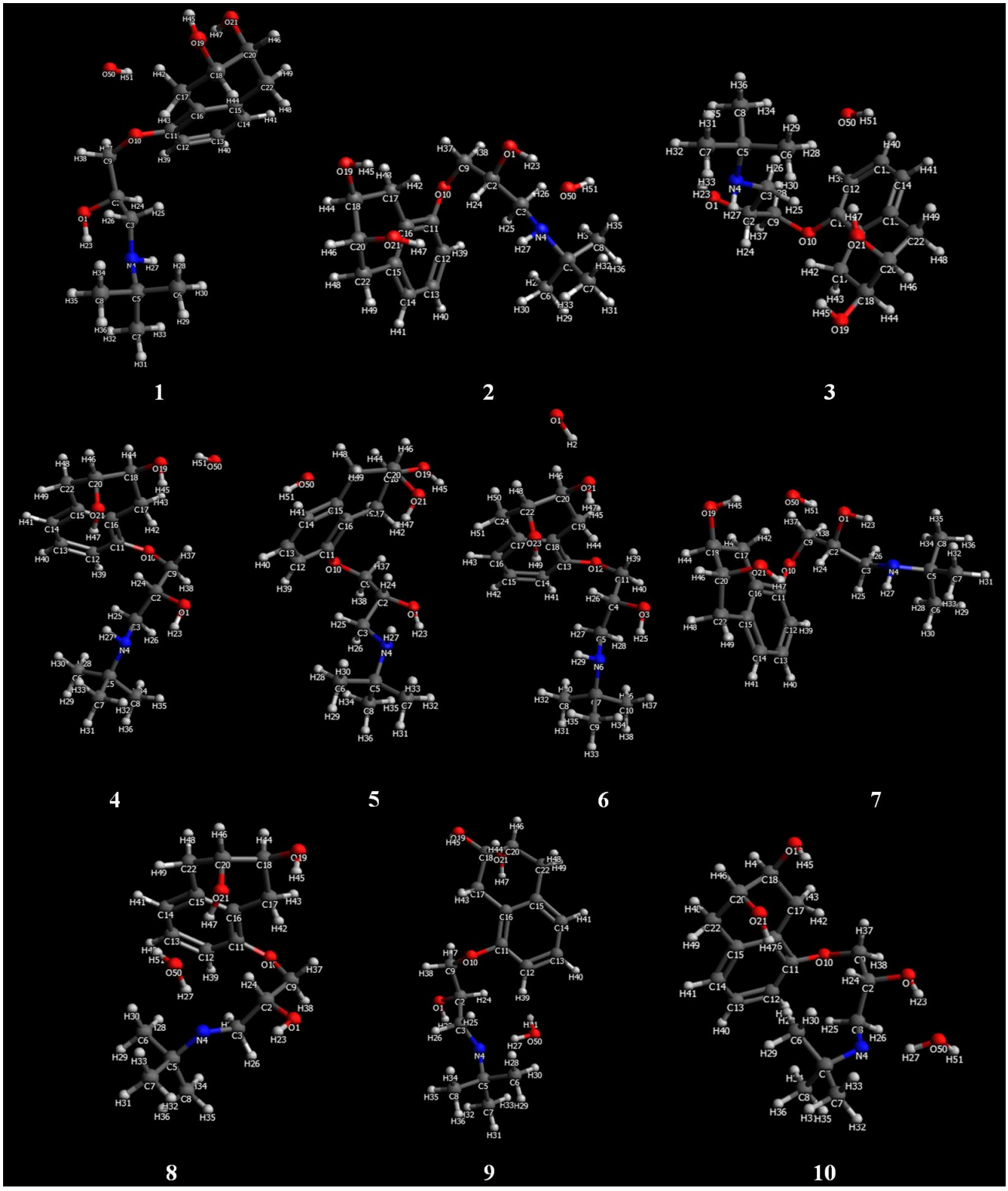


Figure S2. Optimized geometries of NAD/˙OH system

Table S3a. NMR chemical shifts of NAD's degradation intermediate 1.

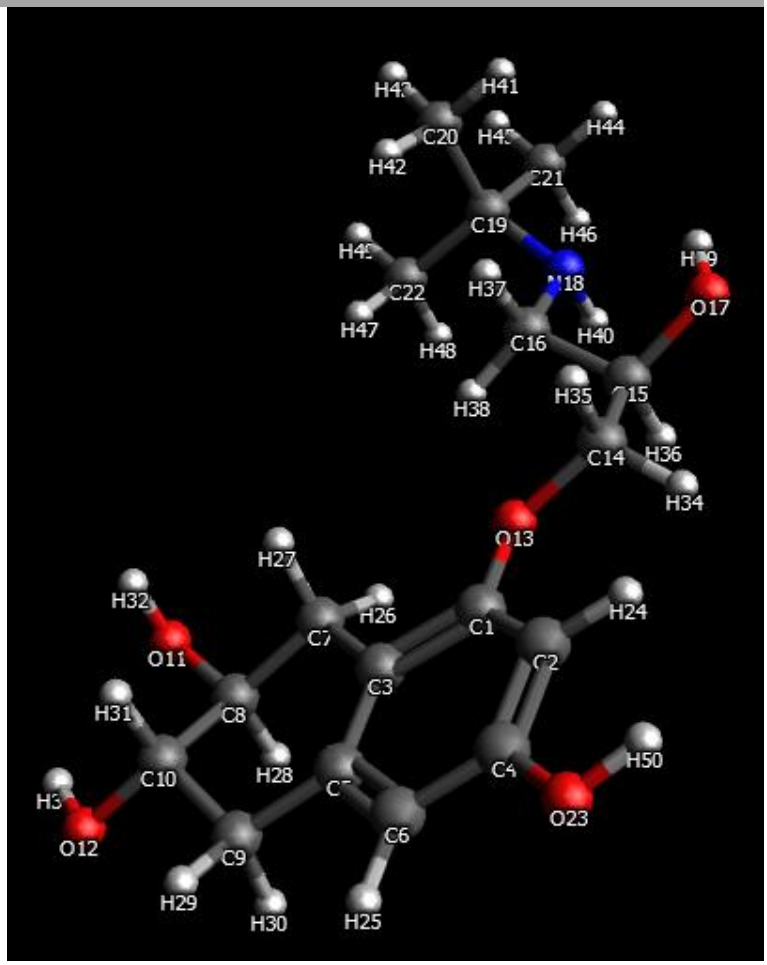
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 1
C1	160.24	**H24**	8.25	
C2	96.45	**H25**	6.51	
C3	116.44	**H26**	4.56	
C4	156.84	**H27**	3.38	
C5	138.34	**H28**	4.31	
C6	112	**H29**	3.32	
C7	34.39	**H30**	3.36	
C8	79.14	**H31**	3.68	
C9	42.66	**H32**	1.53	
C10	77.57	**H33**	3.33	
O11	N/A	**H34**	5.74	
O12	N/A	**H35**	4.7	
O13	N/A	**H36**	5.48	
C14	70.51	**H37**	5.74	
C15	72.97	**H38**	6.97	
C16	50.05	**H39**	3.73	
O17	N/A	**H40**	1.91	
N18	N/A	**H41**	0.79	
C19	51.62	**H42**	3.65	
C20	22.93	**H43**	0.66	
C21	26.51	**H44**	-1.65	
C22	22.47	**H45**	-2.48	
O23	N/A	**H46**	-2.13	
		H47	4.7	
		H48	1.44	
		H49	0.93	
		H50	5.56	

Table S3b. NMR chemical shifts of NAD's degradation intermediate 2.

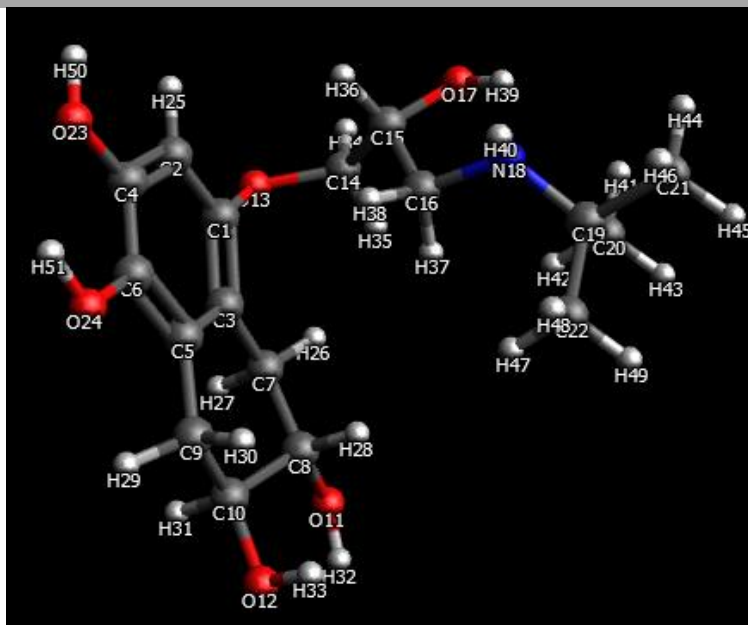
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 2
C1	150.58	**H25**	7.42	
C2	109.27	**H26**	3.35	
C3	122.49	**H27**	2.52	
C4	143.57	**H28**	4.28	
C5	125.22	**H29**	3.18	
C6	141.41	**H30**	2.98	
C7	37.38	**H31**	3.68	
C8	75.33	**H32**	3.45	
C9	34.04	**H33**	1.17	
C10	77.45	**H34**	5.07	
O11	N/A	**H35**	4.85	
O12	N/A	**H36**	3.88	
O13	N/A	**H37**	3.85	
C14	87.17	**H38**	3.07	
C15	68.55	**H39**	5.17	
C16	51.17	**H40**	3.49	
O17	N/A	**H41**	0.47	
N18	N/A	**H42**	2.47	
C19	52.46	**H43**	0.29	
C20	24.78	**H44**	-0.81	
C21	27.19	**H45**	-1.5	
C22	21.58	**H46**	-0.69	
O23	N/A	**H47**	3.48	
O24	N/A	**H48**	2.26	
		H49	1.12	
		H50	5.09	
		H51	6.12	

Table S3c. NMR chemical shifts of NAD's degradation intermediate 3.

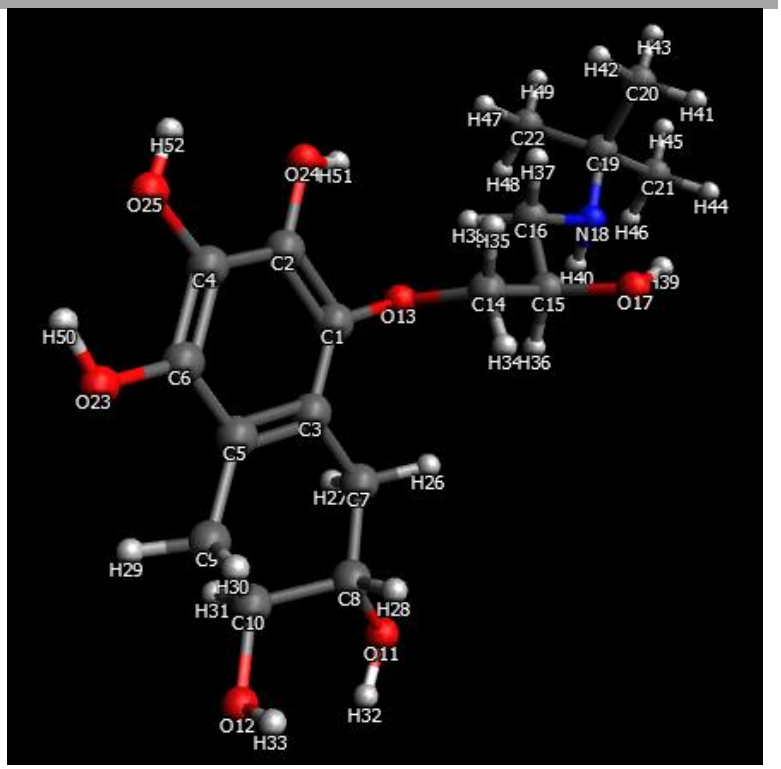
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 3
C1	142.38	**H26**	3.74	
C2	140.4	**H27**	2.7	
C3	121.31	**H28**	4.09	
C4	134.67	**H29**	3.13	
C5	114.49	**H30**	2.84	
C6	141.96	**H31**	3.74	
C7	36.11	**H32**	3.48	
C8	75.14	**H33**	1.16	
C9	35.61	**H34**	4.62	
C10	77.69	**H35**	5.96	
O11	N/A	**H36**	6.66	
O12	N/A	**H37**	7.01	
O13	N/A	**H38**	7.49	
C14	80.33	**H39**	5.68	
C15	74.23	**H40**	5.21	
C16	51.45	**H41**	0.62	
O17	N/A	**H42**	2.29	
N18	N/A	**H43**	-1.35	
C19	51.39	**H44**	-0.54	
C20	21.85	**H45**	-2.84	
C21	26.6	**H46**	-0.78	
C22	23.11	**H47**	3.25	
O23	N/A	**H48**	1.73	
O24	N/A	**H49**	-0.85	
O25	N/A	**H50**	6.43	
		H51	7.11	
		H52	6.54	

Table S3d. NMR chemical shifts of NAD's degradation intermediate 4.

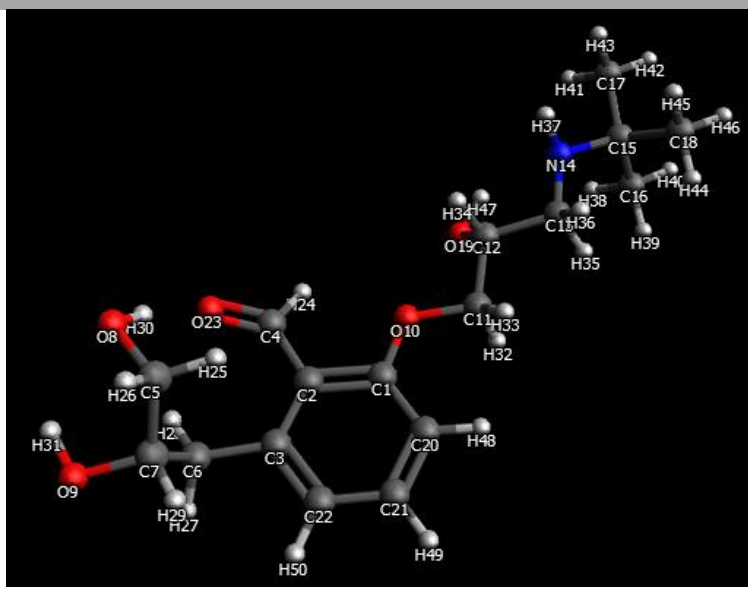
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 4
C1	169.46	**H24**	11.35	
C2	123.96	**H25**	1.44	
C3	145.48	**H26**	3.3	
C4	204.34	**H27**	3.55	
C5	69.78	**H28**	3.68	
C6	40.76	**H29**	3.13	
C7	77.76	**H30**	3.99	
O8	N/A	**H31**	5.11	
O9	N/A	**H32**	7.29	
O10	N/A	**H33**	8.63	
C11	76.59	**H34**	8.98	
C12	75.25	**H35**	7.91	
C13	50.69	**H36**	8.23	
N14	N/A	**H37**	6.7	
C15	47.52	**H38**	-0.28	
C16	18.8	**H39**	1.11	
C17	23.79	**H40**	-3.95	
C18	20.97	**H41**	-1.5	
O19	N/A	**H42**	-5.55	
C20	110.4	**H43**	-1.92	
C21	138.35	**H44**	1.91	
C22	124.02	**H45**	0.66	
O23	N/A	**H46**	-3.61	
		H47	6.75	
		H48	9.53	
		H49	8.68	
		H50	7.26	

Table S3e. NMR chemical shifts of NAD's degradation intermediate 5.

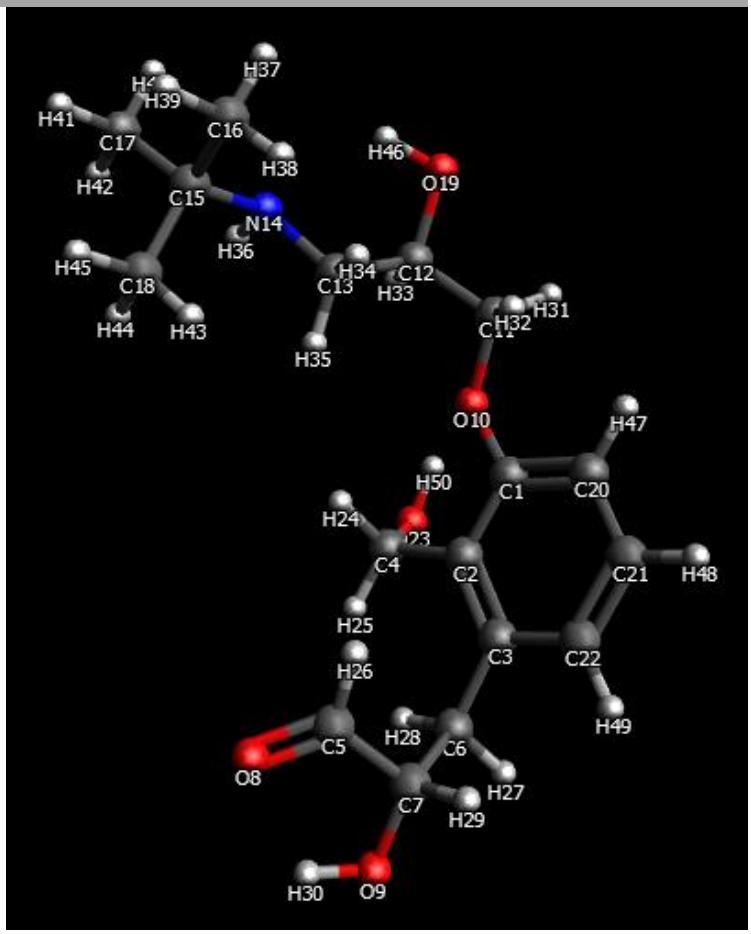
Label	Relative chemical shift			Structure of intermediate 5
C1	163.52	**H24**	6.14	
C2	131.86	**H25**	5.4	
C3	138.36	**H26**	10.24	
C4	62.84	**H27**	3.66	
C5	215.67	**H28**	3.49	
C6	38.38	**H29**	5.08	
C7	83.99	**H30**	4.06	
O8	N/A	**H31**	5.55	
O9	N/A	**H32**	6.07	
O10	N/A	**H33**	4.89	
C11	72.78	**H34**	6.77	
C12	74.28	**H35**	7.31	
C13	52.74	**H36**	1.95	
N14	N/A	**H37**	-0.05	
C15	51.14	**H38**	3.49	
C16	23.03	**H39**	0.19	
C17	25.46	**H40**	-2.38	
C18	23.4	**H41**	-2.8	
O19	N/A	**H42**	-2.25	
C20	111.51	**H43**	5.18	
C21	131.04	**H44**	2.22	
C22	123.35	**H45**	1.24	
O23	N/A	**H46**	3.21	
		H47	8.95	
		H48	8.7	
		H49	8.55	
		H50	3.27	

Table S3f. NMR chemical shifts of NAD's degradation intermediate 6.

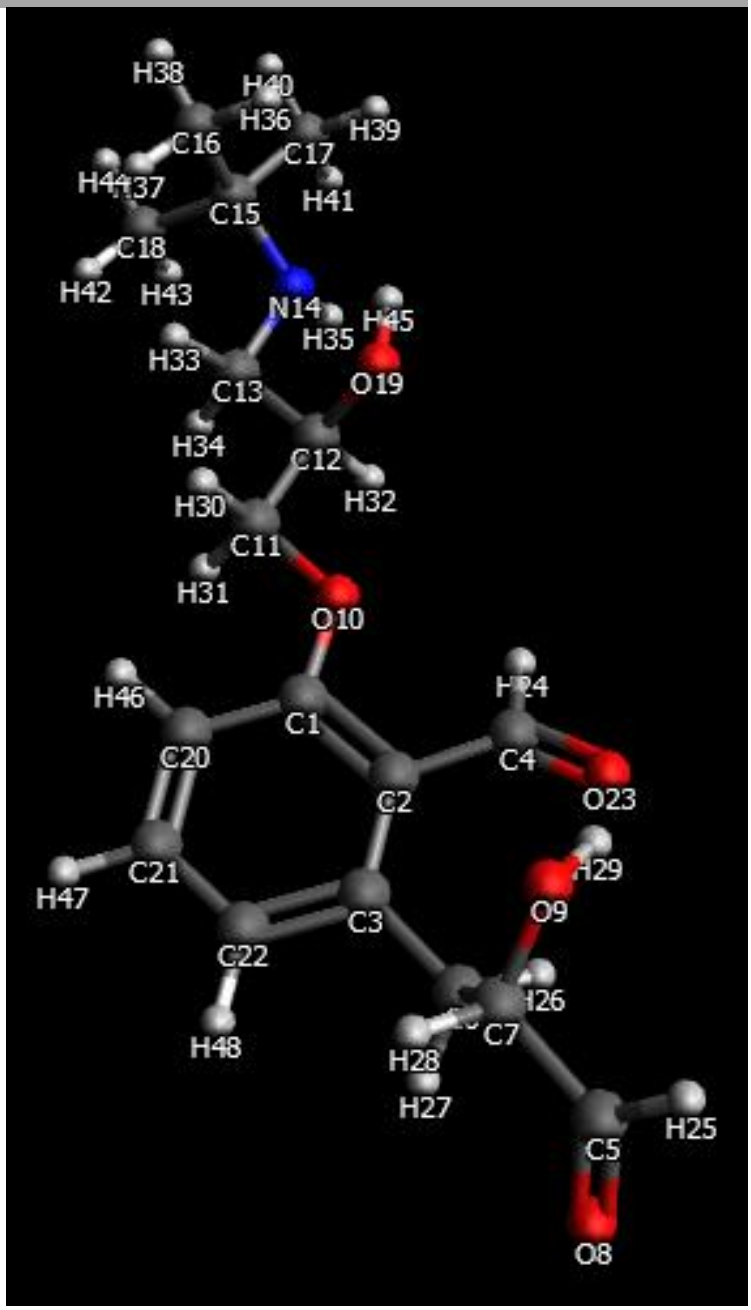
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 6
C1	167.66	**H24**	11.41	
C2	122.39	**H25**	12.23	
C3	145.51	**H26**	2.15	
C4	202.81	**H27**	1.55	
C5	221.62	**H28**	3.58	
C6	38.81	**H29**	5.62	
C7	86.81	**H30**	7.73	
O8	N/A	**H31**	8.77	
O9	N/A	**H32**	8.33	
O10	N/A	**H33**	8.67	
C11	79.02	**H34**	8.42	
C12	74.72	**H35**	5.8	
C13	52.11	**H36**	-0.15	
N14	N/A	**H37**	2.12	
C15	47.33	**H38**	-3.17	
C16	19.5	**H39**	-2.19	
C17	23.06	**H40**	-6.01	
C18	20.73	**H41**	-2.91	
O19	N/A	**H42**	2.68	
C20	111.38	**H44**	-3.12	
C21	138.85	**H45**	6.22	
C22	123.76	**H46**	9.93	
O23	N/A	**H47**	8.66	
		H48	6.94	

Table S3g. NMR chemical shifts of NAD's degradation intermediate 7.

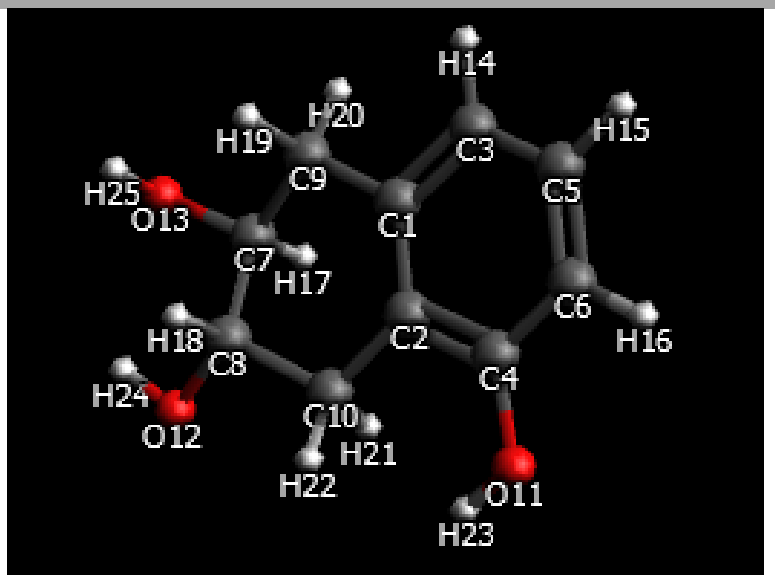
Label	Relative chemical shift	Structure of intermediate 7
C1	140.53	
C2	121.87	
C3	121.86	
C4	156.72	
C5	128.28	
C6	116.01	
C7	76.04	
C8	74.93	
C9	34.84	
C10	27.4	
O11	N/A	
O12	N/A	
O13	N/A	
H14	7.19	
H15	7.74	
H16	6.88	
H17	4.05	
H18	4.29	
H19	3.36	
H20	3.14	
H21	2.61	
H22	3.38	
H23	5.8	
H24	2.54	
H25	0.27	

Table S3h. NMR chemical shifts of NAD's degradation intermediate 8.

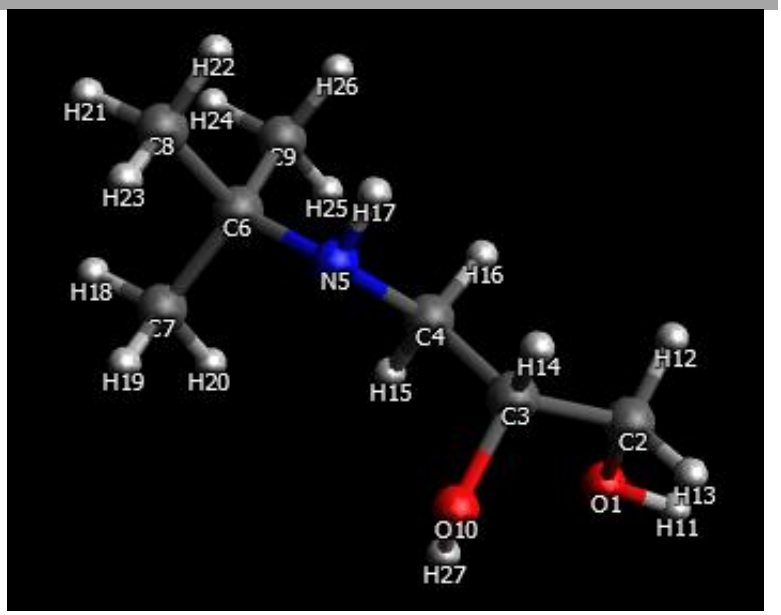
Label	Relative chemical shift	Structure of intermediate 8
O1	N/A	
C2	68.24	
C3	80.07	
C4	54.36	
N5	N/A	
C6	55.42	
C7	26.4	
C8	31.22	
C9	25.8	
O10	N/A	
H11	1.32	
H12	3.64	
H13	4.46	
H14	2.76	
H15	2.85	
H16	2.79	
H17	0.67	
H18	2.04	
H19	1.37	
H20	2.23	
H21	1.52	
H22	0.36	
H23	0.51	
H24	1.33	
H25	1.46	
H26	0.54	
H27	2.06	

Table S3i. NMR chemical shifts of NAD's degradation intermediate 9.

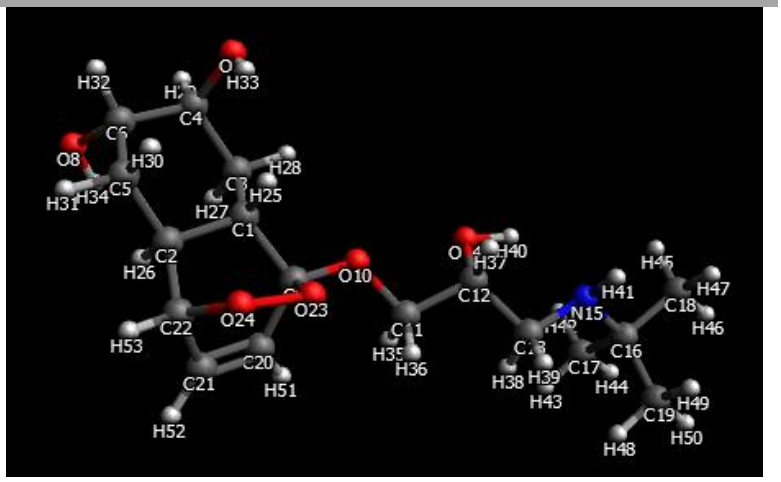
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 9
C1	41.2	**H25**	0.99	
C2	44.98	**H26**	1.13	
C3	34.76	**H27**	1.66	
C4	77.1	**H28**	2.25	
C5	31.99	**H29**	4	
C6	77.47	**H30**	1.81	
O7	N/A	**H31**	1.84	
O8	N/A	**H32**	3.83	
C9	108.78	**H33**	2.28	
O10	N/A	**H34**	1.92	
C11	78.98	**H35**	6.95	
C12	72.45	**H36**	7.08	
C13	47.05	**H37**	7.45	
O14	N/A	**H38**	8.08	
N15	N/A	**H39**	7.45	
C16	49.79	**H40**	8.38	
C17	21.07	**H41**	5.91	
C18	24.92	**H42**	2.75	
C19	21.66	**H43**	3.27	
C20	130.66	**H44**	-0.63	
C21	138.45	**H45**	0.91	
C22	78.51	**H46**	-3.21	
O23	N/A	**H47**	-1.4	
O24	N/A	**H48**	1.54	
		H49	-0.7	
		H50	-2.86	
		H51	8.43	
		H52	7.93	
		H53	4.68	

Table S3j. NMR chemical shifts of NAD's degradation intermediate 10.

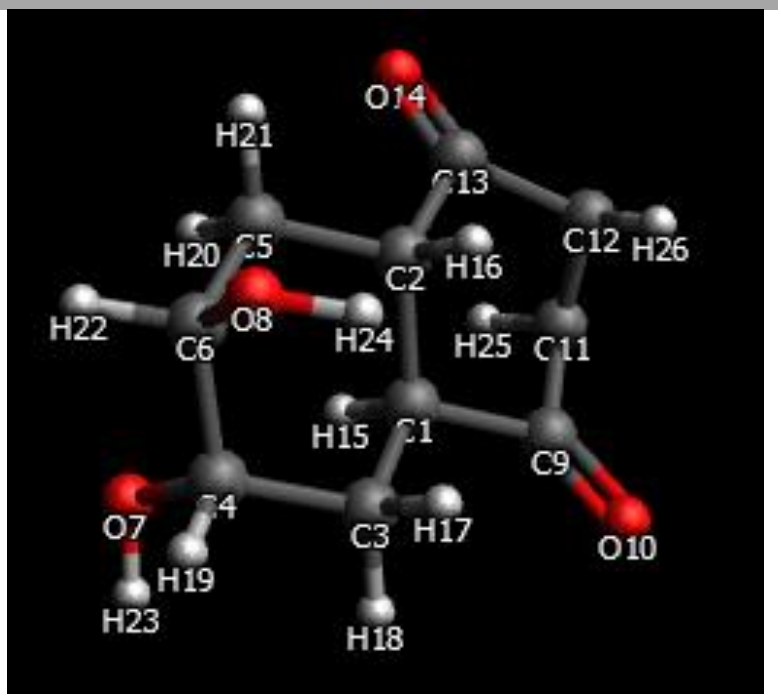
Label	Relative chemical shift	Structure of intermediate 10
C1	51.61	
C2	51	
C3	26.17	
C4	73.78	
C5	33.49	
C6	76.62	
O7	N/A	
O8	N/A	
C9	210.5	
O10	N/A	
C11	139.07	
C12	139.72	
C13	210.13	
O14	N/A	
H15	4.42	
H16	3.77	
H17	2.35	
H18	2.76	
H19	2.79	
H20	2.43	
H21	1.93	
H22	2.62	
H23	1.05	
H24	1.3	
H25	7.81	
H26	7.71	

Table S3k. NMR chemical shifts of NAD's degradation intermediate 11.

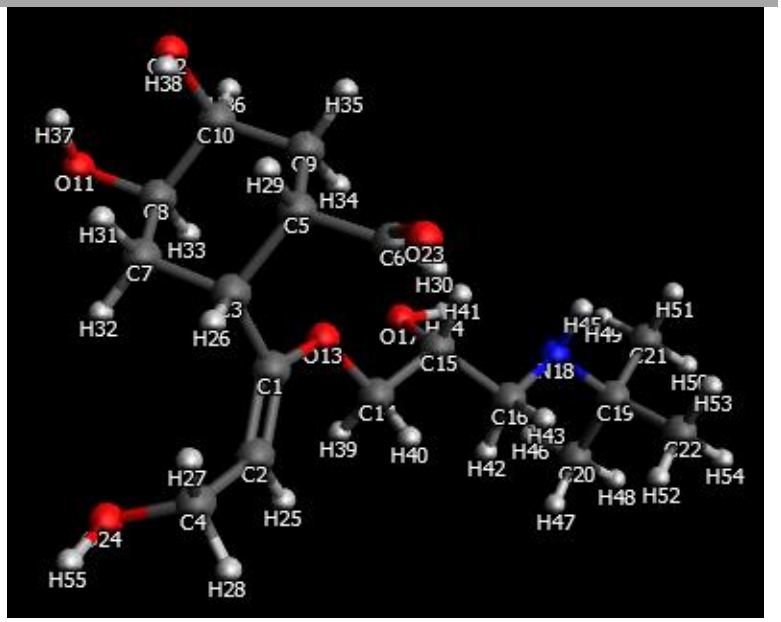
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 11
C1	171.65	**H25**	7.12	
C2	102.87	**H26**	4.26	
C3	39.04	**H27**	4.85	
C4	59.79	**H28**	5.45	
C5	48.59	**H29**	3.23	
C6	218.83	**H30**	11.68	
C7	38.27	**H31**	2.63	
C8	72.87	**H32**	2.27	
C9	28.12	**H33**	4.64	
C10	75.9	**H34**	4.2	
O11	N/A	**H35**	2.79	
O12	N/A	**H36**	5.03	
O13	N/A	**H37**	2.96	
C14	80	**H38**	2.37	
C15	76.98	**H39**	6.96	
C16	50.87	**H40**	7.81	
O17	N/A	**H41**	8.29	
N18	N/A	**H42**	7.54	
C19	49.46	**H43**	7.9	
C20	19.49	**H44**	6.4	
C21	25.24	**H45**	5.23	
C22	21.57	**H46**	0.69	
O23	N/A	**H47**	1.84	
O24	N/A	**H48**	-2.44	
		H49	-0.92	
		H50	-4.46	
		H51	-1.81	
		H52	2.31	
		H53	0.3	
		H54	-2.69	
		H55	1.71	

Table S3l. NMR chemical shifts of NAD's degradation intermediate 12.

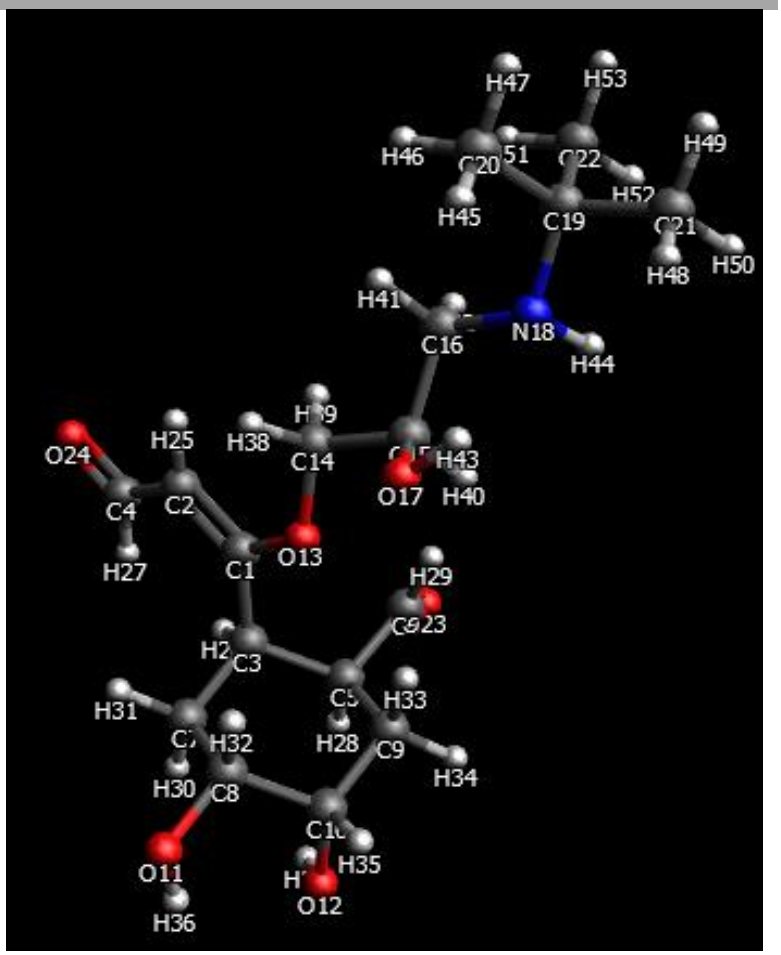
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 12
C1	194.68	**H25**	9.44	
C2	110.28	**H26**	7.34	
C3	39.12	**H27**	11.71	
C4	198.23	**H28**	4.82	
C5	49.17	**H29**	13.4	
C6	217.93	**H30**	4.14	
C7	34.03	**H31**	3.83	
C8	71.96	**H32**	5.92	
C9	25.7	**H33**	5.11	
C10	75.6	**H34**	2.5	
O11	N/A	**H35**	4.23	
O12	N/A	**H36**	2.18	
O13	N/A	**H37**	2.44	
C14	80.51	**H38**	9.01	
C15	76.41	**H39**	10.71	
C16	52.1	**H40**	9.65	
O17	N/A	**H41**	8.74	
N18	N/A	**H42**	9.25	
C19	47.17	**H43**	6.85	
C20	18.8	**H44**	5.45	
C21	22.07	**H45**	0.28	
C22	20.35	**H46**	2.13	
O23	N/A	**H47**	-3.41	
O24	N/A	**H48**	-2.29	
		H49	-6.5	
		H50	-3.22	
		H51	2.53	
		H52	-0.01	
		H53	-3.69	

Table S3m. NMR chemical shifts of NAD's degradation intermediate 13.

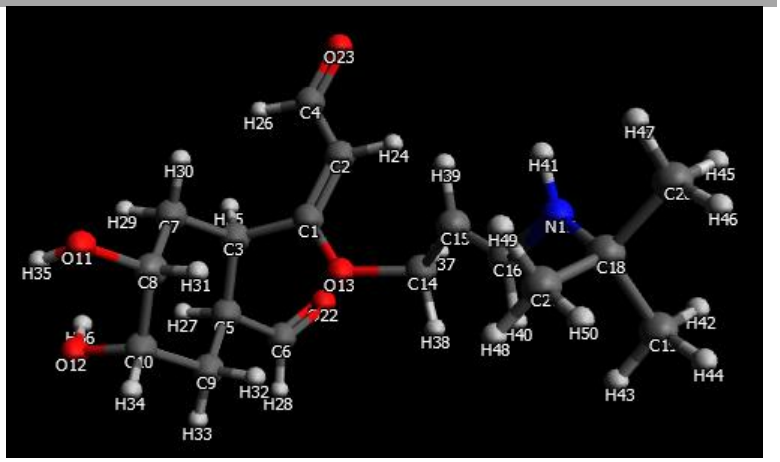
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 13
C1	194.31	**H24**	7.05	
C2	109.04	**H25**	5.73	
C3	35.15	**H26**	10.43	
C4	197.96	**H27**	4.3	
C5	48.76	**H28**	10	
C6	209.76	**H29**	3.65	
C7	31.85	**H30**	3.1	
C8	71.65	**H31**	5.67	
C9	23	**H32**	3.48	
C10	75.61	**H33**	1.79	
O11	N/A	**H34**	3.85	
O12	N/A	**H35**	2.01	
O13	N/A	**H36**	2.06	
C14	77.89	**H37**	6.65	
C15	90.87	**H38**	7.49	
C16	143.91	**H39**	7.82	
N17	N/A	**H40**	11.21	
C18	51.07	**H41**	9.17	
C19	18.3	**H42**	0.38	
C20	24.4	**H43**	1.83	
C21	23.97	**H44**	-1.81	
O22	N/A	**H45**	-0.19	
O23	N/A	**H46**	-2.87	
		H47	0.25	
		H48	3.69	
		H49	2.86	
		H50	-0.45	

Table S3n. NMR chemical shifts of NAD's degradation intermediate 14.

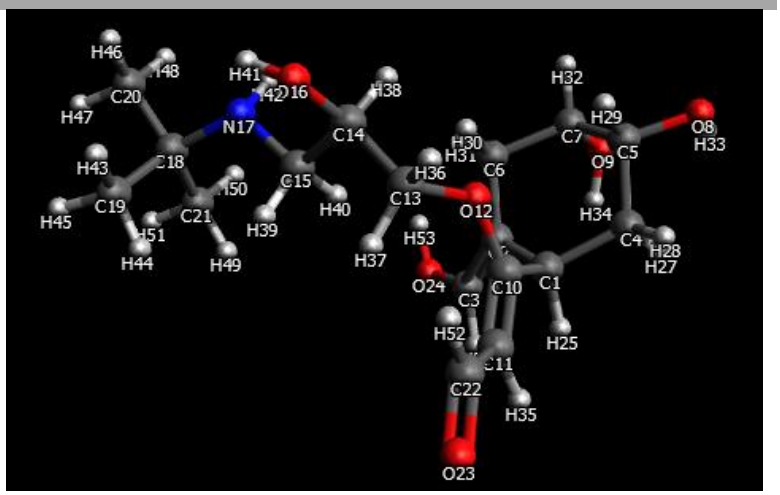
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 14
C1	48.82	**H25**	6.08	
C2	117.14	**H26**	10.84	
C3	146.58	**H27**	2.66	
C4	35.97	**H28**	1.69	
C5	71.99	**H29**	3.26	
C6	21.78	**H30**	5.21	
C7	74.59	**H31**	4.79	
O8	N/A	**H32**	2.96	
O9	N/A	**H33**	1.78	
C10	192.91	**H34**	4.52	
C11	110.14	**H35**	6.58	
O12	N/A	**H36**	5.41	
C13	83.56	**H37**	5.71	
C14	74.94	**H38**	4.51	
C15	53.63	**H39**	6.39	
O16	N/A	**H40**	9.16	
N17	N/A	**H41**	1.54	
C18	55	**H42**	3.32	
C19	22.96	**H43**	-1.3	
C20	25.72	**H44**	2.55	
C21	24.44	**H45**	-0.18	
C22	199.84	**H46**	-2.99	
O23	N/A	**H47**	-1.99	
O24	N/A	**H48**	-0.72	
		H49	7.45	
		H50	5.44	
		H51	3.53	
		H52	10.87	
		H53	8.54	

Table S3o. NMR chemical shifts of NAD's degradation intermediate 15.

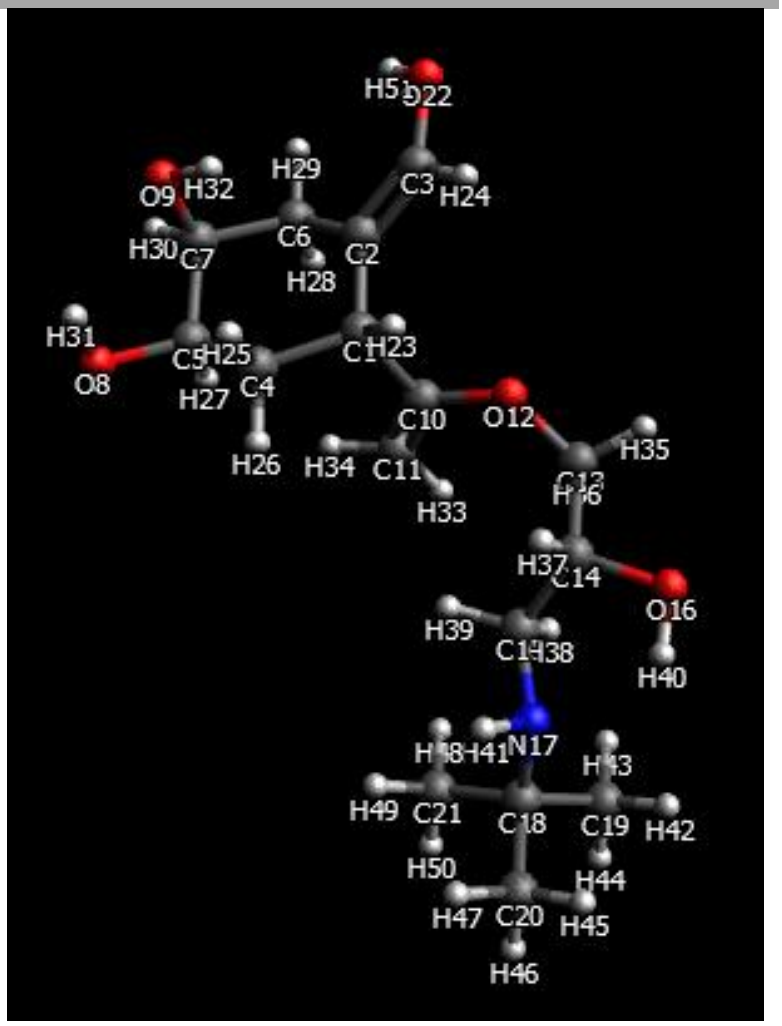
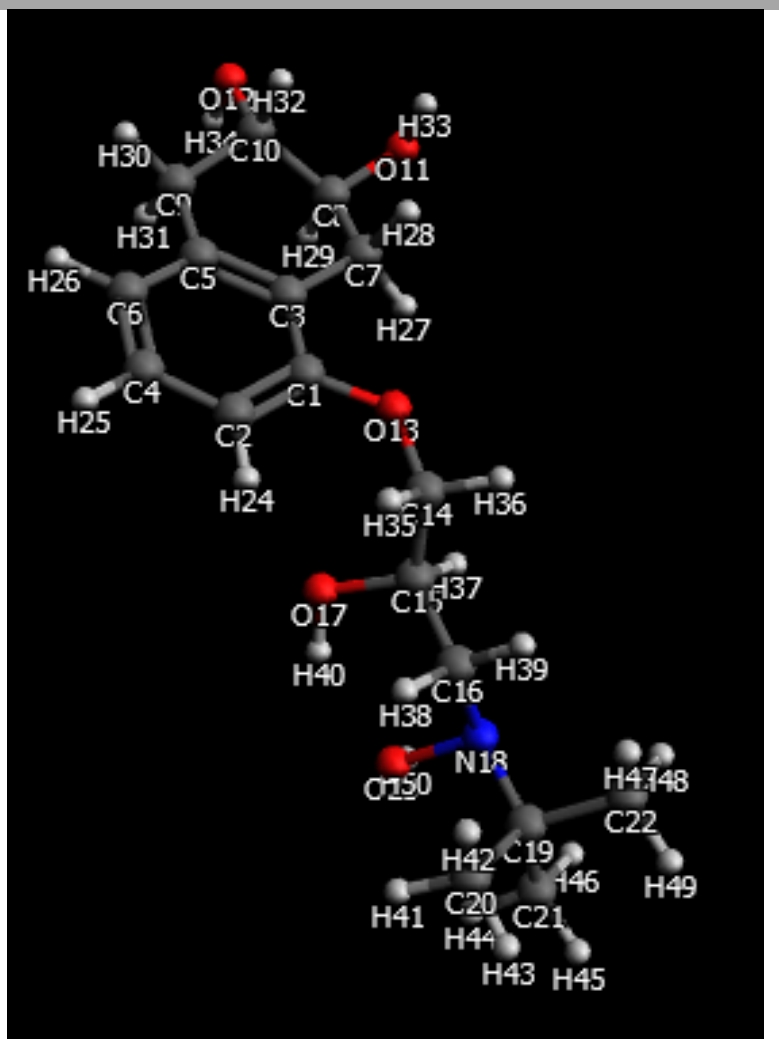
Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 15
C1	49.17	**H23**	3.49	
C2	107.19	**H24**	6.88	
C3	147.83	**H25**	2.81	
C4	34.78	**H26**	3.27	
C5	73.16	**H27**	5.11	
C6	20.86	**H28**	2.37	
C7	73.98	**H29**	2.99	
O8	N/A	**H30**	4.39	
O9	N/A	**H31**	2.68	
C10	169.45	**H32**	2.49	
C11	83.04	**H33**	5.68	
O12	N/A	**H34**	5.4	
C13	75.7	**H35**	4.47	
C14	78.04	**H36**	5.33	
C15	57.7	**H37**	4.62	
O16	N/A	**H38**	4.99	
N17	N/A	**H39**	5.61	
C18	52.75	**H40**	3.96	
C19	22.8	**H41**	4.78	
C20	28.92	**H42**	-0.74	
C21	24.37	**H43**	0.92	
O22	N/A	**H44**	-1.46	
		H45	-0.36	
		H46	-0.95	
		H47	1.02	
		H48	3.32	
		H49	3.51	
		H50	0.92	
		H51	4.61	

Table S3p. NMR chemical shifts of NAD's degradation intermediate 16.

Label	Relative chemical shift	Label	Relative chemical shift	Structure of intermediate 16
C1	159.18	**H24**	8.21	
C2	110.6	**H25**	7.78	
C3	121.94	**H26**	6.58	
C4	127.22	**H27**	3.44	
C5	135.08	**H28**	3.08	
C6	122.12	**H29**	2.78	
C7	37.85	**H30**	2.89	
C8	78.58	**H31**	1.69	
C9	40.79	**H32**	3.8	
C10	76.23	**H33**	1.5	
O11	N/A	**H34**	0.67	
O12	N/A	**H35**	7.38	
O13	N/A	**H36**	7.19	
C14	66.86	**H37**	7.21	
C15	81.14	**H38**	6.52	
C16	55.23	**H39**	7	
O17	N/A	**H40**	6.21	
N18	N/A	**H41**	2.28	
C19	64.75	**H42**	1.21	
C20	9.53	**H43**	-1.05	
C21	20.86	**H44**	0.95	
C22	18.97	**H45**	-1.87	
O23	N/A	**H46**	0.4	
		H47	0.46	
		H48	1.05	
		H49	-1.41	
		H50	7.65	