Electronic Supplementary Material (ESI) for Metallomics.

Is hydroxypyridonate 3,4,3-LI(1,2-HOPO) a good competitor of fetuin for uranyl metabolism?

Supplementary Information

I. Determination of apparent affinity constant

For each HOPO concentration, the free part of UO_2^{2+} has been quantified by measuring the corresponding SPR signals in duplicates. From these signals, and thanks to a calibration curve (SPR signal = f (UO_2^{2+}) previously established within the same experiment, the unbound UO_2^{2+} could be calculated. A binding isotherm has been obtained by plotting the response SPR signal (Resonance Units, RU) corresponding to the unbound UO_2^{2+} versus the ligand concentration (here log [HOPO]). This gives a sigmoid curve that could be fitted to a four parameter logistic curve to calculate the apparent K_D. These parameters are the maximum response corresponding to 50 nM UO_2^{2+}), the minimum response corresponding to highest HOPO concentration leading to total UO_2^{2+} consumption, the slope and the inflexion point (see Table below and reference [15] for experimental details). This point corresponds to 50 % of UO_2^{2+} occupancy, thus giving the apparent K_D.

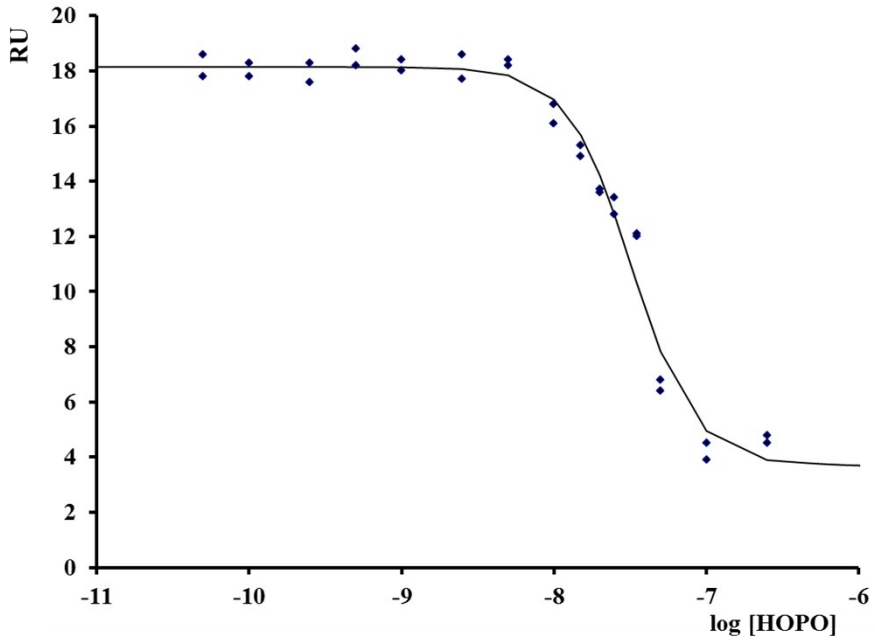

Figure S1. Determination of the apparent K_D of HOPO for UO_2^{2+} by SPR. Uranyl ($0.5x10^{-7}$ M) in TRIS buffer and Na_2CO_3 ($5x10^{-6}$ M) was contacted with varied HOPO concentrations from 0.05 to $250x10^{-9}$ M and in duplicates (diamonds). The curve of the binding isotherm (continuous black line) is fitted to a four parameter logistic curve. The parameters of this curve are given in the table

1 below. The apparent K_D of HOPO is deduced from the HOPO concentration giving 50 % of signal (i.e 50% occupancy).

Table S1. Best fit parameters for the binding isotherm of uranyl with HOPO

MIN (RU)	3.7 ± 0.2	
MAX (RU)	18.1 ± 0.3	**K_D (50% signal)**
Inflexion point (log)	-7.49 ± 0.03	**$32 \pm 0.3 \times 10^{-9}$ M**
Slope	2.07	

II. Titration of HOPO by uranyl; UV-Vis spectroscopy

In order to further define the stoichiometry of the UO_2^{2+}-HOPO complex, titration of HOPO by uranyl has been performed at 22 ± 2 °C. The evolution of the HOPO UV-Vis spectra is presented (Figure S2) within the 250-500 nm range. The aquo-ion of UO_2^{2+} has a negligible absorption between within this range. HOPO at pH 7.4 presents a maximum absorbance at λ = 328 nm (ε = 20.3 $L.mol^{-1}.cm^{-1}$).

Changes on the UV-Vis spectra were followed for successive additions of 0.1 equivalents of UO_2^{2+} from 0 up to 2 UO_2^{2+} equivalents, and for additions of 0.5 equivalents up to 4 equivalents. The first successive additions from 0 to 2 UO_2^{2+} equivalents led to a decrease of the 328 nm signal associated with a blue shift of the wavelength from 328 to 317 nm for 1 equivalent, and then to 314 nm for 2 equivalents. The ligand band at 328 nm is blue shifted upon the successive UO_2^{2+} addition because the π - π^* transition is affected by the complexation with uranyl. After this 2:1 ratio, the absorption increase at 314 and 328 nm remain proportional to UO_2^{2+} additions without modification of the wavelength maximum.

The ratio of absorbance at 328 over 314 nm decreases with a single slope up to 2 UO_2^{2+} equivalents where a clear breakpoint is observed, followed then by a plateau. (Figure S2, insert). Knowingly, HOPO is an octadentate chelator composed of a spermine backbone coupled to four hydroxypyridinone groups for metal binding and the nature of UO_2^{2+}-ligand complex formation comes to occupy in equatorial plane, that define HOPO as having four potential bidentate hydroxypyridinone rings for uranyl complexation.

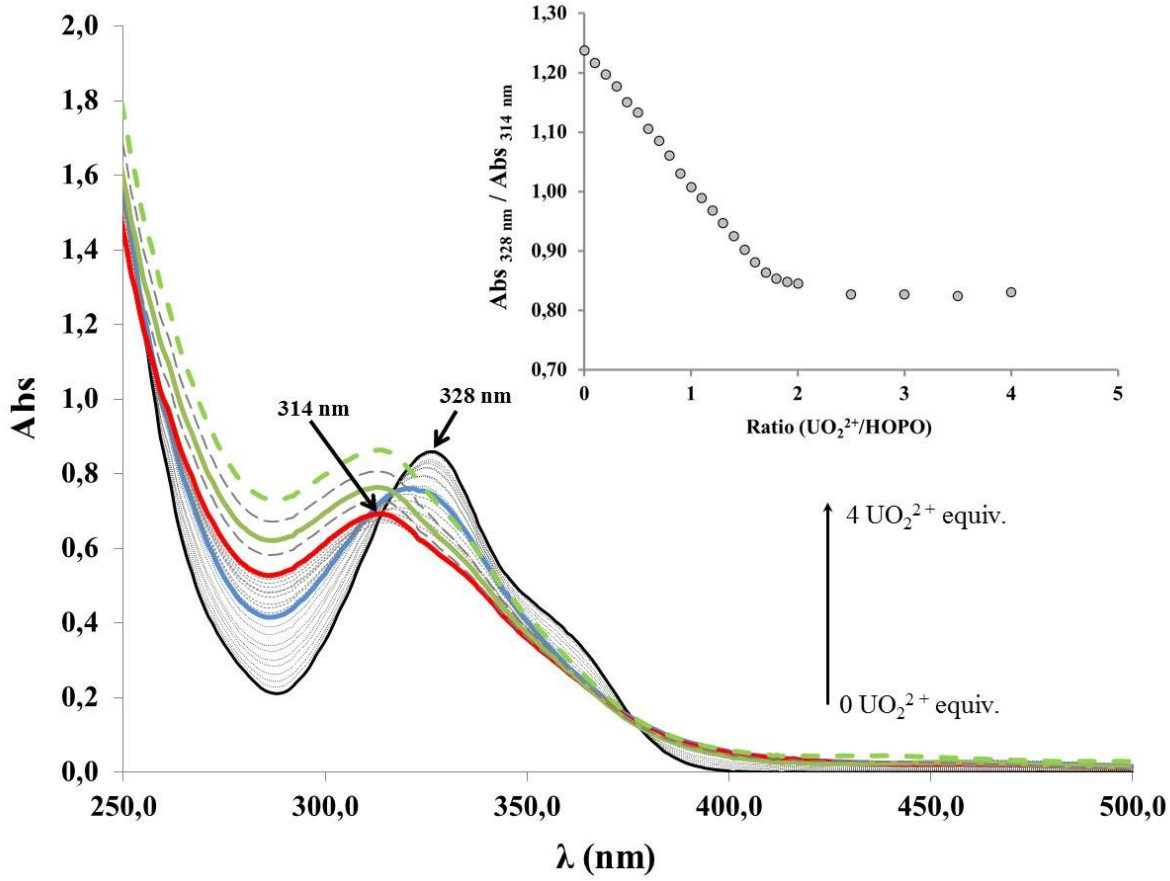

Figure S2. UV-vis titration of HOPO by UO_2^{2+}. Evolution of HOPO spectra ($5x10^{-5}$ M in TRIS buffer at 22 ± 2 °C upon UO_2^{2+} additions. Successive additions of 0.1 UO_2^{2+} equivalents from 0 (continuous black line) to 1 (continuous blue line) and 2 equivalents (continuous red line); then additions of 0.5 equivalents from 2 to 3 (continuous green line) and 4 equivalents (dashed green line). Insert: evolution of the absorbance ratio at 328 vs 314 nm (wavelengths corresponding to the 2 black arrows on the spectra) upon UO_2^{2+} additions.

III. EXAFS data analysis

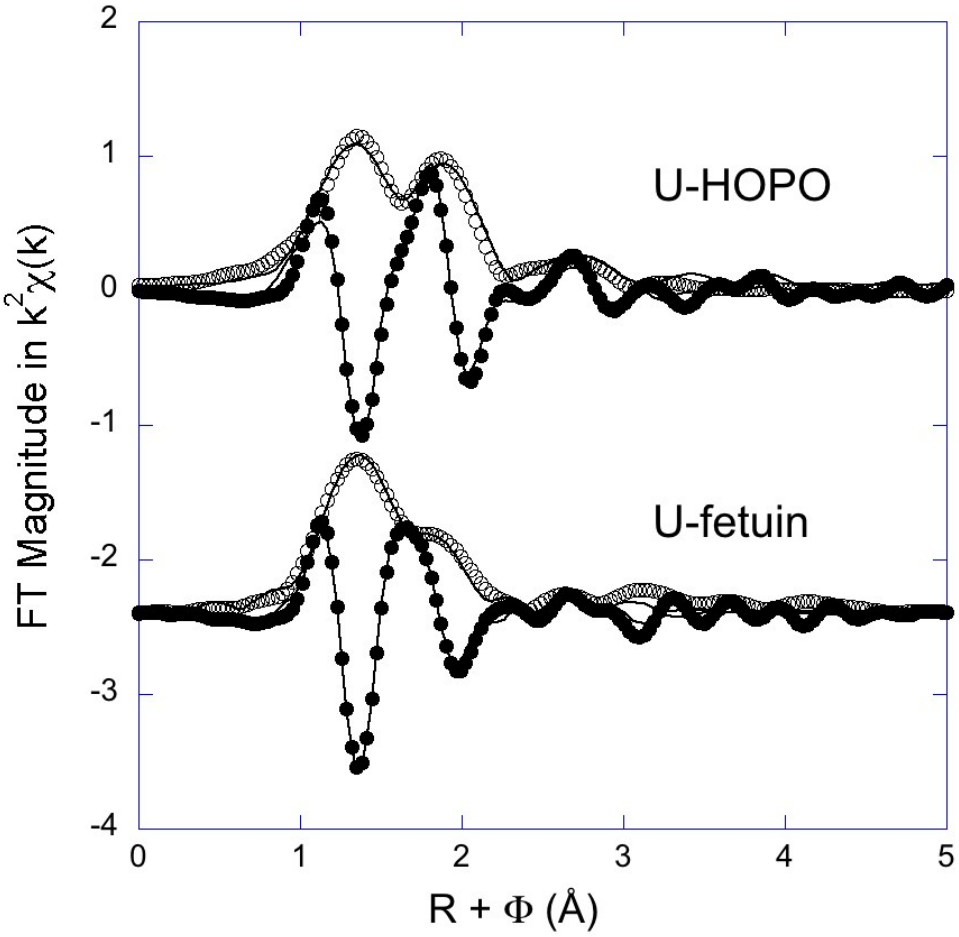

Figure S3. Moduli and imaginary parts of the Fourier tranforms of the EXAFS spectra of UO_2^{2+}-HOPO and UO_2^{2+}-fetuin (U- for clarity in the Figure) at pH = 6.0. Black curve = experiment, black dots = fit of the imaginary part, empty dots = fit of the modulus.

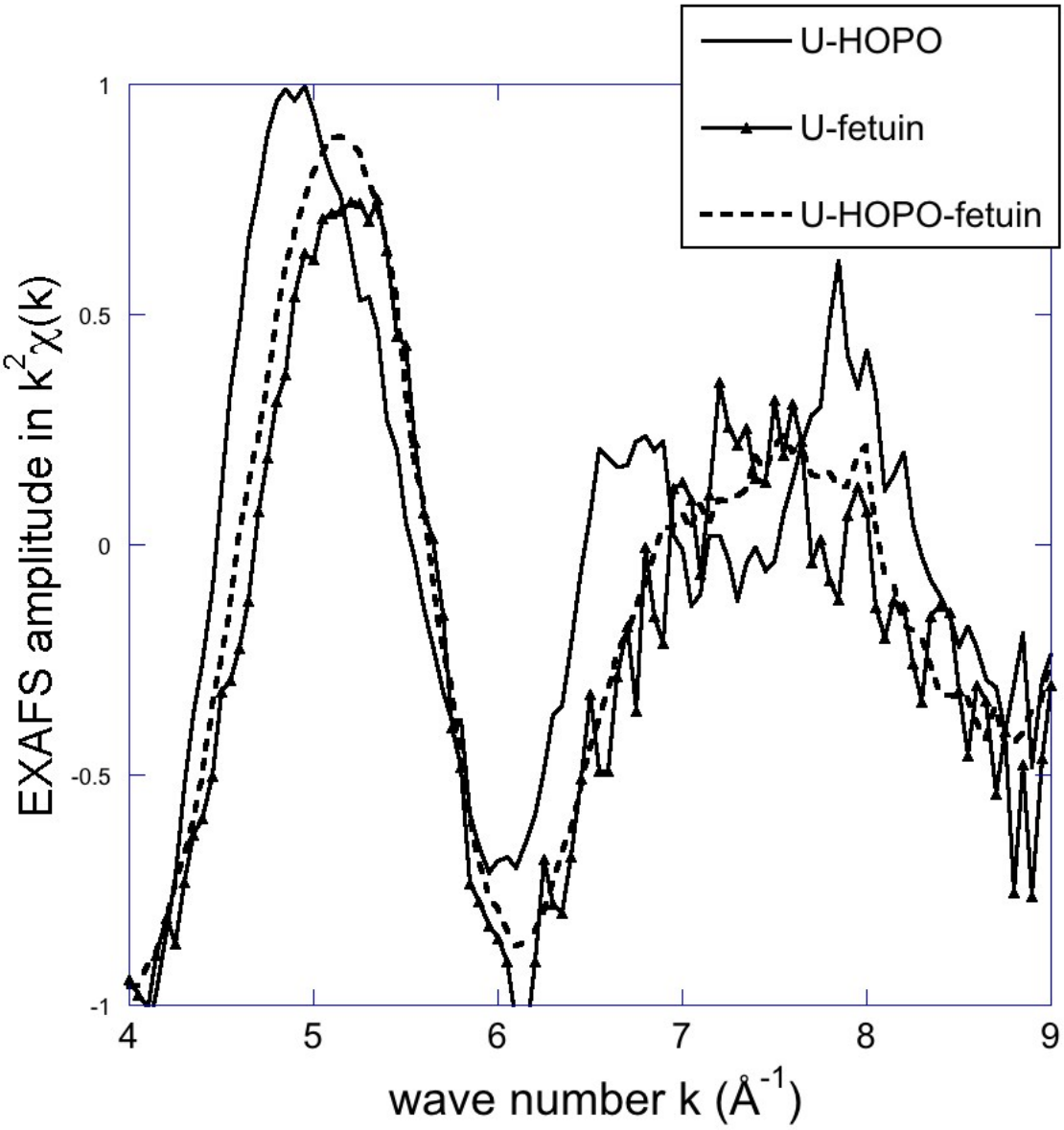

Figure S4. Comparison between the EXAFS spectra of U-HOPO, U-fetuin and U-HOPO-fetuin (enlarged spectral area between 4 and 9 $Å^{-1}$).

IV. DFT calculations

Table S2. xyz coordinates of the two calculated structures presented in Fig. 3 (in Å)

- Without water molecule:

C	2.08799200	1.58084900	3.51463700
C	1.63408600	2.15470100	4.68312700
C	0.80984800	1.39287300	5.55370900
C	0.47790300	0.08977400	5.25779800
C	0.94760500	-0.55540600	4.06864200
N	1.72688100	0.28486500	3.23406900
H	0.44648700	1.84500800	6.46917500
H	1.93524100	3.16229300	4.93381800
H	-0.13757800	-0.50680700	5.91868500
O	2.29907600	-0.29669000	2.06035100
O	0.72307600	-1.75999500	3.70878000
C	3.07532200	2.30823100	2.62658800
O	4.24170700	2.49848900	3.10597400
N	2.71132600	2.78994500	1.41649500
C	1.32514300	2.73974800	0.89068900
H	1.40020400	2.71993900	-0.20057000
H	0.86147300	1.79459400	1.17705800
C	-4.22008000	-2.13835500	1.26281300
C	-4.61027900	-3.17719900	2.10187600
C	-3.84681600	-4.35625100	2.18168900
C	-2.68647900	-4.48809900	1.43371200
C	-2.27536300	-3.43748500	0.59020700
N	-3.07359300	-2.30560700	0.51831700
H	-4.16032100	-5.15798900	2.83863900
H	-5.50595500	-3.03557100	2.68814300
H	-2.06234100	-5.36998500	1.48201200
O	-2.61330300	-1.33908400	-0.38832100
O	-1.20006100	-3.41323500	-0.15570300
C	-5.04847700	-0.88152300	1.23058400
O	-6.06047100	-0.81676200	2.00749600
N	-4.70168600	0.11615100	0.39197000
H	-3.86150600	-0.02822200	-0.17743000
C	-5.47308500	1.36851400	0.32649900
H	-5.45517000	1.71862400	-0.70757500
H	-6.50356200	1.11778100	0.58293400
C	-1.91391600	2.80677700	-2.41409000
C	-1.50737100	3.75268100	-3.34750200
C	-0.37820600	3.50238400	-4.15374800
C	0.32947300	2.31696800	-4.01932700
C	-0.08849700	1.34595900	-3.08104500
N	-1.23463200	1.62363100	-2.33507100
H	-0.06174500	4.24130600	-4.87990600
H	-2.07345700	4.66918100	-3.43790200
H	1.20194100	2.09444300	-4.61898800
O	-1.65683100	0.60649900	-1.47236500
O	0.47991200	0.19816900	-2.82683200
C	-3.12166800	3.08517300	-1.55917000
O	-4.21241900	3.34134400	-2.17445700
N	-3.01084000	3.17087400	-0.21020400

C	-4.18957200	3.61567400	0.58109600
H	-3.83205300	4.33820400	1.32124700
H	-4.85804400	4.14074100	-0.10184400
C	4.45487100	-1.62706800	-0.79939000
C	5.31520900	-2.65703100	-0.43448500
C	4.82147800	-3.96332600	-0.25771700
C	3.47094100	-4.23399700	-0.43697500
C	2.58831700	-3.19648600	-0.80357700
N	3.12706300	-1.92972500	-0.98983300
H	5.49696100	-4.75913900	0.03157600
H	6.35634600	-2.41342400	-0.28079000
H	3.05727500	-5.22339900	-0.29443400
O	2.20262000	-0.98032500	-1.43700100
O	1.29720500	-3.29303100	-1.00478600
C	4.98634000	-0.22625300	-0.94213300
O	6.24690700	-0.04081800	-0.86631800
N	4.10418900	0.78128800	-1.12268000
H	3.13139900	0.49930600	-1.28241700
C	4.55221900	2.17087900	-1.31553300
H	3.78832200	2.68759800	-1.90329200
H	5.46665700	2.14053000	-1.91491600
C	-4.95570800	2.47988300	1.28273200
H	-4.34121900	2.02233200	2.06735000
H	-5.80705800	2.94581600	1.79419500
C	-1.75128300	2.94882600	0.54568000
H	-2.04455800	2.75616400	1.58187200
H	-1.28551100	2.03179000	0.18051000
C	-0.79406500	4.15345200	0.49538100
H	-1.31424500	5.05165500	0.85068000
H	-0.51401500	4.35464900	-0.54687200
C	0.47922500	3.94981400	1.33923700
H	0.22507300	3.83902900	2.40147900
H	1.08552700	4.86070700	1.26047800
C	3.69007800	3.65795500	0.69856800
H	4.12112100	4.35322700	1.42460900
H	3.11355200	4.24586000	-0.02024700
C	4.85647200	2.93557200	-0.00758200
H	5.35820700	2.26317500	0.69433600
H	5.58140700	3.72323800	-0.25225800
U	-0.27323700	-1.46010000	-1.24617300
O	-0.84197815	-2.19961017	-2.79955712
O	0.21744205	-0.80597229	0.38090782
H	1.53450100	-0.64265900	1.50074700

- With two water molecules

C	1.82058100	-1.37471800	2.67562900
C	1.75038500	-1.36351800	4.04884200
C	0.73385700	-2.11604000	4.69568600
C	-0.16262900	-2.86653700	3.97087400
C	-0.11251100	-2.92566700	2.53809200
N	0.90697200	-2.12418500	1.97460000
H	0.67265500	-2.10076400	5.77746400
H	2.46592900	-0.78389400	4.61530800
H	-0.93272400	-3.45447200	4.45252200
O	1.11475500	-2.20357400	0.56397400
O	-0.87836200	-3.59962000	1.78042500

C	2.93703500	-0.68843000	1.92274000
O	4.08693600	-1.22117500	1.97513400
N	2.67962200	0.47418700	1.28192700
C	1.34986400	1.13920900	1.28053300
H	1.25144300	1.63702200	0.31143200
H	0.57891500	0.37053800	1.31997200
C	-5.43088000	-1.47502900	1.36024300
C	-6.09957900	-2.62395000	1.76079900
C	-5.93234100	-3.83063500	1.05729500
C	-5.09585700	-3.88811100	-0.04668100
C	-4.40630100	-2.72925200	-0.45477500
N	-4.59892000	-1.57007800	0.26678800
H	-6.46321400	-4.71738600	1.38050000
H	-6.74964100	-2.55415000	2.62014900
H	-4.94671800	-4.79699800	-0.61285500
O	-3.87394500	-0.47247200	-0.22218900
O	-3.59175600	-2.62809600	-1.47116300
C	-5.66431900	-0.19277600	2.11309300
O	-6.41178000	-0.23438000	3.14177800
N	-5.10775700	0.94544100	1.65733800
H	-4.48943500	0.87404600	0.84499300
C	-5.35797700	2.24065600	2.31123500
H	-5.34605500	3.00321500	1.53019500
H	-6.36229300	2.20407300	2.73507000
C	-1.89729400	3.75546800	-0.55543000
C	-1.22155000	4.86423900	-1.04542600
C	-0.37570100	4.73142000	-2.16237200
C	-0.22253800	3.49914700	-2.78218600
C	-0.92735400	2.38262600	-2.29328500
N	-1.74962300	2.56384600	-1.20158300
H	0.15925000	5.59576300	-2.53541500
H	-1.34662700	5.81615500	-0.54821800
H	0.42226500	3.36169000	-3.63862800
O	-2.44323100	1.42464200	-0.77681200
O	-0.88457000	1.16294900	-2.77458900
C	-2.82520400	3.90179900	0.62269700
O	-3.81629000	4.68426800	0.47969000
N	-2.51466600	3.29888700	1.79596900
C	-3.29515900	3.64975000	3.01268700
H	-2.57863000	3.80693000	3.82410700
H	-3.79650500	4.59645000	2.81198300
C	8.40024000	0.37909000	-0.39194600
C	9.24718100	1.47223900	-0.25934900
C	10.56864900	1.28950300	0.18154300
C	11.02537700	0.02101300	0.48212000
C	10.19518500	-1.13322400	0.35794100
N	8.87046400	-0.88992700	-0.09350600
H	11.22669600	2.14499400	0.28661300
H	8.85447400	2.44862500	-0.50142800
H	12.03864400	-0.14885100	0.82483600
O	8.05047700	-2.02016600	-0.23459300
O	10.57710800	-2.33124000	0.62312700
C	6.98152200	0.64571900	-0.84620900
O	6.66862900	1.83660400	-1.18588800
N	6.14248900	-0.40420000	-0.85846000
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C	4.70891400	-0.44645700	-1.20462200

H	4.29826000	-1.27746300	-0.62564500
H	4.61235700	-0.70932300	-2.26614400
C	-4.32321700	2.57919800	3.41887700
H	-3.81382600	1.65912300	3.72666400
H	-4.84428200	2.95704800	4.30666000
C	-1.34942600	2.38916000	1.97915200
H	-1.57678100	1.77523700	2.85490600
H	-1.31499900	1.71324300	1.12473300
C	-0.01165500	3.12519500	2.19173300
H	-0.09797400	3.79450900	3.05550800
H	0.20506500	3.75436200	1.32064900
C	1.16758400	2.15364400	2.43027300
H	1.01638900	1.60702500	3.36924000
H	2.08660900	2.73853700	2.54934800
C	3.77968500	1.19385500	0.58174200
H	4.71187700	0.98695700	1.10862300
H	3.57205800	2.26189700	0.67822500
C	3.87944900	0.81491500	-0.91153100
H	4.29873900	1.66433300	-1.45041200
H	2.87081700	0.63802400	-1.30505400
U	-2.23867700	-0.72724400	-1.99620500
O	-3.38531000	-0.29403700	-3.32506400
O	-1.06425475	-1.27390174	-0.71483477
H	0.24086100	-1.97673600	0.11811900
O	-1.76601000	-2.96951500	-3.21627900
H	-1.97566600	-2.98549200	-4.16901700
H	-2.02012600	-3.81316200	-2.79681200
O	-0.30897700	-1.02934100	-3.56895500
H	0.14133600	-0.20072300	-3.81566900
H	0.25023700	-1.82429400	-3.60598300