Efficient Removal of Uranium from the Kidney by a Picolinate-based Pentadentate Chelator

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Materials

Caution! $^{238}UO_2(NO_3)_2 \cdot 6H_2O$ is an alpha-emitter, and all relative operations adhered to standard handling procedures for radioactive materials. The ligand 6,6'-[azanediylbis(methylene)] dipicolinic acid (**H_2dpa**) and picolinic acid (**Pic**) were purchased from Wuxi AppTec and Aladdin Scientific, respectively. All other chemicals were obtained from commercial sources and used without further purification.

Characterization methods

NMR measurements were conducted at 298 K on an Avance NEO-400 from Bruker. Chemical shifts for ^{1}H are reported in ppm on the δ scale; ^{1}H signals are referenced to the residual solvent peak: dimethyl sulfoxide (DMSO-d_6) (2.500 ppm). Inductively coupled plasma mass spectrometry (ICP-MS) was recorded on Agilent 7900 mass spectrometer. UV-Vis spectra were measured on a Shimadzu UV-2600 spectrophotometer using quartz cuvettes with an optical path length of 1 cm.

Ligand characterization

The NMR spectra of **H_2dpa** and **Pic** are consistent with those reported in the previous literature.[1,2]

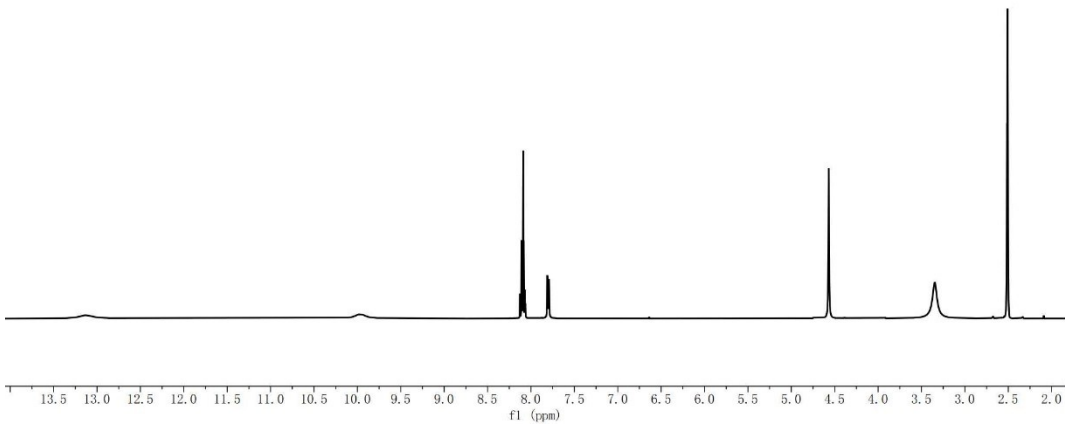

Figure S1 ^{1}H NMR spectrum (400 MHz, 298 K, DMSO-d_6) of **H_2dpa**.

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 8.14 – 8.03 (m, 4H), 7.79 (dd, J = 7.0, 1.8 Hz, 2H), 4.56 (s, 4H).

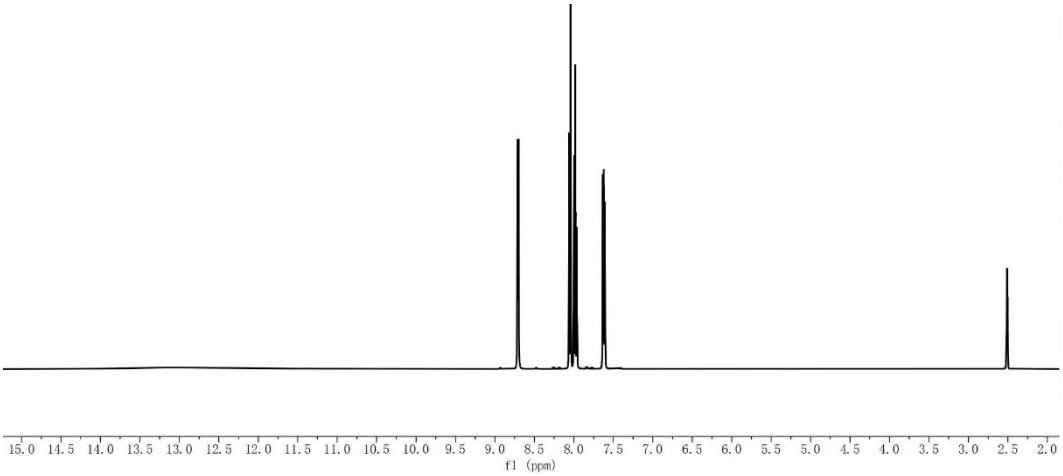

Figure S2 ^{1}H NMR spectrum (400 MHz, 298 K, DMSO-d_6) of **Pic**.

^{1}H NMR (400 MHz, DMSO-d_6): δ (ppm) = 8.70 (ddd, J = 4.7, 1.7, 0.9 Hz, 1H), 8.04 (dt, J = 7.9, 1.2 Hz, 1H), 7.97 (td, J = 7.7, 1.7 Hz, 1H), 7.61 (ddd, J = 7.5, 4.7, 1.3 Hz, 1H).

Computational details

The calculation was performed using the ORCA 5.0.4 program.[3–6] The geometry optimization and frequency calculations were carried out at B3LYP-D3(BJ)/SDD/ma-def2-TZVP(-f) level.[7–11] The Stuttgart/Dresden relativistic effective core potential (SDD, 60 core electrons) and associated valence basis set (32 valence electrons) were applied for the U atom.[12,13] Solvent effects were considered using the Conductor-like Polarizable Continuum Model (CPCM) solvent model.[14] Electronic energies were calculated at PWPB95-D3(BJ)/SARC-DKH-TZVPP/ma-DKH-def2-TZVPP.[15,16] When U was treated with the SARC-DKH-TZVPP basis set, the Coulomb-fitting basis sets employed SARC/J, whereas in other cases the auxiliary basis sets were generated automatically by ORCA for other elements.[17,18] The scalar relativistic effect was considered using the Douglas–Kroll–Hess Hamiltonian of second order (DKH2).[19,20] Gibbs free energy changes (ΔG) of hypothetical complexation reactions were calculated by $\Delta G = \Sigma G(\text{products}) - \Sigma G(\text{reactants})$, $G = \varepsilon_e + \Delta G_{0\rightarrow T} + \Delta G_{solv}$ + 1.89 kcal/mol. $\Delta G_{solv} = E_{soln} - E_{gas}$, E_{soln} and E_{gas} are the energies in liquid and gas phases, which is calculated at M06-2X/SDD/6-31G* level.[21,22] The solvation model based on density (SMD) was used to calculate E_{soln}.[23] $\Delta G_{0\rightarrow T}$ was calculated using the Shermo application with Grimme's mRRHO model.[24,25]1.89 kcal/mol is an energy correction term for a

molecule from 1 atm to 1 mol/L. For water, the term for a molecule from 1 atm to liquid (55.6mol/L) is 4.27 kcal/mol. In cases of multiple **Pic** binding with uranyl, the standard state concentration of **Pic** is 2 mol/L or 3 mol/L. The Gibbs free energy of a proton in aqueous solution, as reported in the literature, is -270.29 kcal/mol (1M).[26,27]

Table S1 Calculated ΔG values of six hypothetical complexation reactions.

Reactions	ΔG (kcal/mol)
$2Pic^{-}+UO_2(H_2O)_5^{2+}\rightarrow UO_2(Pic)_2(H_2O)+4H_2O$ [a]	-42.17
$3Pic^{-}+UO_2(H_2O)_5^{2+}\rightarrow UO_2(Pic)_3^{-}+5H_2O$ [b]	-52.72
$Hdpa^{-}+UO_2(H_2O)_5^{2+}\rightarrow UO_2dpa+5H_2O+H^{+}$ [c]	-59.59
$dpa^{2-}+UO_2(H_2O)_5^{2+}\rightarrow UO_2dpa+5H_2O$ [c]	-60.35
$Hdpa^{-}+UO_2(H_2O)_5^{2+}\rightarrow UO_2dpa(H_2O)+4H_2O+H^{+}$ [d]	-54.12
$dpa^{2-}+UO_2(H_2O)_5^{2+}\rightarrow UO_2dpa(H_2O)+4H_2O$ [d]	-54.88

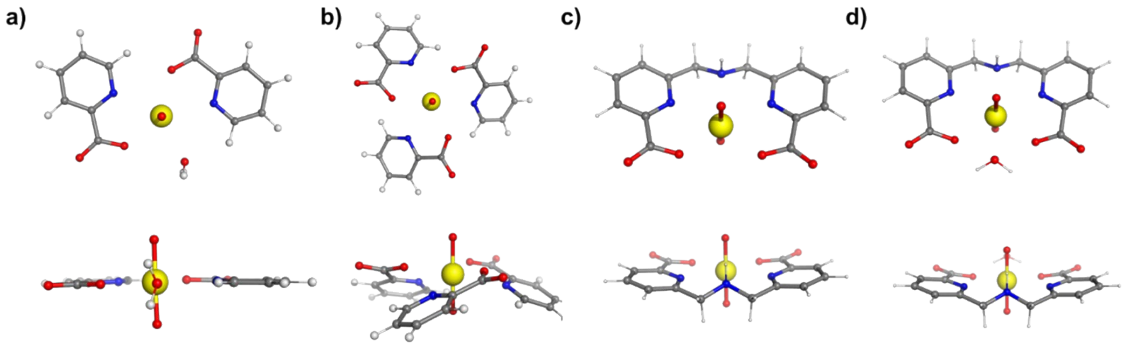

Figure S3 Top and side views of optimized structures of a) UO_2(**Pic**)$_2(H_2O)$, b) UO_2(**Pic**)$_3^{-}$, c) UO_2**dpa**, and d) UO_2**dpa**(H_2O).

Determination of conditional stability constants

Determined by Job's plot analysis, the metal-ligand stoichiometry of uranyl complexes herein is 1:1 for H_2dpa. The calculation of the conditional binding constant was treated and fitted based on spectrophotometric titration data with the HypSpec program.[28] Titration data ranging from 260 to 375 nm were employed to refine the conditional stability constant.

Cytotoxicity assays of uranyl and chelating agents

Cells in log phase were cultured in a 96-well plate for 24 h at a seeding density of 3000 cells per well in 0.1 mL of DMEM/F-12, and 0.1 mL culture medium contained 12.4 μM U(VI) and different concentrations of chelating agents. After 48 h, 10.0 μL of CCK-8 was added to every well, and the cells were cultured for 1–2 h. The absorbance spectrum of every well was obtained by a Synergy Neo Multi-Mode Reader (BioTek). The cell survival rate was calculated using the formula: [OD (experimental group) - OD (blank)] / [OD (control group) - OD (blank)] ×100%. Each group of the above assays was performed with six parallel samples. In this assay, only the culture medium was added to the wells of the control group.

Table S2 Dosage-dependent cell growth rate of NRK-52E cells treated with 12.4 μM U(VI) + $ZnNa_3DTPA$, 12.4 μM U(VI) + **Pic** and 12.4 μM U(VI) + **H_2dpa**.

Concentration (μM)	U(VI) + $ZnNa_3DTPA$ Survival Rate (%)	U(VI) + **Pic** Survival Rate (%)	U(VI) + **H_2dpa** Survival Rate (%)
50	85.36%±11.92%	95.17%±3.58%	92.64%±14.04%
100	83.18%±16.13%	96.37%±5.50%	89.24%±16.52%
200	84.37%±14.34%	94.60%±4.95%	81.99%±14.18%
400	87.28%±17.26%	92.40%±5.65%	66.18%±16.10%

In vivo uranium decorporation assays

Female Balb/c mice (6-8 weeks old) were obtained for the in vivo uranium decorporation assays. These animal assays were approved by the Animal Care and Use Committee of Soochow University.

U(VI) stock solution ($UO_2(NO_3)_2{\cdot}6H_2O$) was prepared in normal saline (NS); the ligand **Pic**, **H_2dpa** or $ZnNa_3DTPA$ was dissolved in 5.000 mL NS, respectively, to prepare the ligand solution with the additional NaOH as a base to adjust the pH value to 7-8.

Experimental and control groups of five mice each were intravenously (i.v.) injected with 0.2 mL $^{238}U(VI)$ solution as the standard dosage (0.5 mg $^{238}U(VI)$ kg^{-1}). For the experimental groups, 0.5 mL ligand solution (molar ratio of ligands to U(VI) = 92:1 for **H_2dpa** and 184:1 for **Pic**) was administered by intraperitoneal (i.p.) injection promptly

after, while the control group was only given 0.5 mL saline. All mice were fed water and food 4 h after ^{238}U injection. Mice were dissected to obtain kidney, femur, liver, spleen, and muscle samples 24 h after the initial injection of U(VI) solution. The tissue samples were digested in aqua regia, and the ^{238}U content in each sample was determined by ICP-MS (Agilent).

Table S3 The uranium removal efficiency of **Pic** and **H_2dpa** with single dosage prompt injection, all values reported as μg (U) per g (tissue).

Tissue Type	U(VI) + NS	U(VI) + **Pic**	U(VI) + **H_2dpa**
Kidneys	7.165 ±2.415	8.231 ±1.344	0.622 ±0.113
Femurs	1.465 ±0.232	1.327±0.207	1.382±0.178
Liver & Spleen & Muscle	0.030±0.019	0.037±0.015	0.027±0.010

Another experimental assay using the above-mentioned procedures was conducted to compare the in vivo removal efficiency of the clinical drug $ZnNa_3DTPA$ with that of **H_2dpa**. For the experimental groups, 0.5 mL ligand solution (molar ratio of ligands to U(VI) = 184:1) was administered by intraperitoneal (i.p.) injection, while the control group was only given 0.5 mL saline.

Table S4 The uranium removal efficiency of $ZnNa_3DTPA$ and **H_2dpa** with single dosage prompt injection, all values reported as μg (U) per g (tissue).

Tissue Type	U(VI) + NS	$ZnNa_3DTPA$	U(VI) + **H_2dpa**
Kidneys	12.331±2.268	9.230±1.029	0.879±0.244
Femurs	2.624 ±0.499	2.878±0.863	2.373±0.375
Liver & Spleen & Muscle	0.079±0.048	0.037±0.013	0.086±0.014

Optimized Cartesian Coordinates

UO_2dpa

C	-3.698747	2.487428	-0.152463
C	-4.804129	1.668668	-0.326074
C	-4.639099	0.288125	-0.321042
C	-3.364135	-0.220797	-0.155415
N	-2.296414	0.577692	0.002272
C	-2.447660	1.904916	0.020405
C	-1.217752	2.713997	0.306954
N	-0.000502	2.039592	-0.191611
C	1.217444	2.713655	0.305641
C	2.447074	1.904244	0.018522
C	3.697910	2.486846	-0.155950
N	2.296231	0.576908	0.002585
C	3.364270	-0.221447	-0.154063
C	4.638969	0.287633	-0.321217
C	4.803471	1.668206	-0.328891
C	-3.068809	-1.701762	-0.120606
C	3.069747	-1.702449	-0.115304
O	1.818141	-1.980380	0.062553
O	-1.817166	-1.979900	0.057135
U	0.001005	-0.545746	0.063134
H	-3.795162	3.564285	-0.147855
H	-5.786204	2.101145	-0.463009
H	-5.472609	-0.387232	-0.444433
H	3.794014	3.563738	-0.152918
H	5.472699	-0.387640	-0.443577
H	5.785334	2.100802	-0.466966
O	3.970443	-2.524876	-0.235098
O	-3.969144	-2.524196	-0.242978
H	-0.001014	2.116460	-1.207136

H	1.312128	3.718316	-0.110426
H	-1.312527	3.718859	-0.108606
O	0.003936	-0.552770	-1.720783
O	-0.001872	-0.411313	1.843241
H	-1.120421	2.812391	1.390932
H	1.121035	2.812601	1.389654

UO_2dpa(H_2O)

C	-13.314971	-1.394263	0.990878
C	-14.471752	-1.926680	0.445271
C	-14.403328	-3.142720	-0.222825
C	-13.173198	-3.765072	-0.335826
N	-12.047544	-3.234901	0.166348
C	-12.116183	-2.083706	0.839815
C	-10.851981	-1.624977	1.487339
N	-9.675241	-2.018456	0.693117
C	-8.451768	-1.695692	1.447584
C	-7.238220	-2.223477	0.757429
C	-5.997892	-1.603684	0.869875
N	-7.394642	-3.366332	0.084629
C	-6.318438	-3.956973	-0.456842
C	-5.051623	-3.406378	-0.382688
C	-4.891827	-2.199137	0.285699
C	-13.002002	-5.112591	-0.982044
C	-6.586919	-5.287476	-1.104973
O	-7.815445	-5.660543	-1.022408
O	-11.794046	-5.552545	-0.938457
U	-9.763876	-4.405896	-0.462918
H	-13.329044	-0.458685	1.532482
H	-15.415033	-1.406712	0.545813
H	-15.277672	-3.612434	-0.647993
H	-5.913157	-0.672291	1.412372

H	-4.220009	-3.923376	-0.837657
H	-3.917576	-1.734291	0.356404
O	-5.678021	-5.928440	-1.628730
O	-13.962982	-5.706383	-1.467202
H	-9.672329	-1.447326	-0.150175
H	-8.363436	-0.621069	1.620649
H	-10.872246	-0.546780	1.659720
O	-9.764769	-3.448609	-1.964307
O	-9.755764	-5.190581	1.135552
H	-10.771518	-2.112540	2.462328
H	-8.527264	-2.181244	2.423883
O	-9.848241	-6.342888	-2.390497
H	-10.625955	-6.875480	-2.172394
H	-9.061973	-6.875983	-2.204268

Hdpa$^-$

C	0.797834	-2.250906	-0.568458
C	2.047675	-1.698265	-0.337622
C	2.209259	-0.807523	0.715271
C	1.122471	-0.519100	1.535667
N	-0.074164	-1.078456	1.322365
C	-0.235724	-1.895640	0.287788
C	-1.843707	1.711564	0.416859
C	-2.057835	0.613381	-0.407616
N	-1.290884	0.336399	-1.464203
C	-0.227906	1.107189	-1.708902
C	0.074508	2.212151	-0.912201
C	-0.756715	2.531220	0.149471
C	-1.634290	-2.428085	0.085776
N	-2.621107	-1.515946	0.750288
C	-3.144757	-0.376257	-0.096080
C	1.259737	0.465101	2.703787

O	2.279009	1.201672	2.680519
O	0.357364	0.453653	3.574134
C	0.680281	0.733762	-2.889741
O	0.220954	-0.058582	-3.746100
O	1.820177	1.265942	-2.887901
H	0.618441	-2.918010	-1.400834
H	2.880211	-1.939959	-0.985450
H	3.157091	-0.327735	0.904734
H	-2.500859	1.902973	1.254601
H	0.950675	2.800965	-1.136767
H	-0.552630	3.393128	0.771271
H	-1.743986	-3.405615	0.554809
H	-1.887823	-2.512306	-0.966818
H	-2.122190	-1.127331	1.566809
H	-3.413783	-2.058523	1.092389
H	-3.548380	-0.820324	-1.002019
H	-3.943920	0.080018	0.482648

dpa^{2-}

C	-0.627962	-0.292008	-1.791477
C	0.535349	-1.037489	-1.725350
C	0.580704	-2.134977	-0.875240
C	-0.549620	-2.464101	-0.133561
N	-1.677191	-1.744377	-0.210530
C	-1.715108	-0.671073	-1.003694
C	-2.982170	0.159657	-0.978016
N	-2.871117	1.488809	-0.362244
C	-2.300711	1.495281	0.990039
C	-0.788003	1.415042	1.040394
C	-0.122463	0.449558	1.788424
N	-0.135692	2.326287	0.312058
C	1.200001	2.316399	0.291023

C	1.937946	1.378375	1.012676
C	1.264248	0.429689	1.766619
C	-0.520407	-3.666104	0.823615
C	1.922172	3.365940	-0.566488
O	3.141288	3.543481	-0.304861
O	0.605559	-4.203902	0.997313
H	-0.695993	0.583911	-2.423383
H	1.399331	-0.764241	-2.317959
H	1.473499	-2.732844	-0.775357
H	-0.683675	-0.284739	2.349686
H	3.016280	1.402098	0.968278
H	1.810767	-0.322586	2.321440
O	1.256451	3.950814	-1.455645
O	-1.601352	-4.014908	1.358106
H	-2.303775	2.093153	-0.947853
H	-2.603978	2.425278	1.479311
H	-3.342107	0.310074	-1.997940
H	-3.739044	-0.408417	-0.435373
H	-2.736235	0.672797	1.557962

$UO_2(Pic)_2(H_2O)$

C	10.490999	-13.190205	1.551682
C	9.290709	-12.809808	2.127576
C	8.703433	-11.617584	1.725316
C	9.339861	-10.849500	0.761212
C	10.541486	-11.296164	0.230337
N	11.107478	-12.444823	0.617077
C	11.186790	-14.472137	1.936903
O	12.295805	-14.677520	1.306363
O	10.702363	-15.220230	2.779565
C	15.038408	-11.676762	-2.742168
C	15.929007	-11.258535	-3.716969

C	17.049378	-12.035978	-3.978082
C	17.236866	-13.203753	-3.253264
C	16.298860	-13.557050	-2.293507
N	15.219156	-12.809486	-2.040925
C	13.801252	-10.882765	-2.404177
O	13.082261	-11.405251	-1.464305
O	13.542905	-9.839886	-2.993770
U	13.341346	-13.379159	-0.302606
O	14.928242	-15.287862	-0.071187
H	8.838112	-13.445699	2.873898
H	7.765524	-11.294853	2.157213
H	8.918975	-9.915106	0.417685
H	11.073881	-10.733287	-0.522518
H	15.734650	-10.340599	-4.251563
H	17.762676	-11.735275	-4.733841
H	18.094312	-13.840027	-3.421713
H	16.407968	-14.458882	-1.709227
H	14.684759	-16.169682	-0.389146
H	15.424101	-15.416262	0.750785
O	14.245404	-12.521198	0.967445
O	12.490562	-14.262884	-1.592218

$UO_2(Pic)_3^-$

C	-1.087823	3.300813	0.284682
C	-1.232423	4.625512	-0.102843
C	-0.152202	5.269811	-0.688375
C	1.030726	4.564138	-0.863041
C	1.094124	3.245051	-0.435759
N	0.057060	2.624787	0.131016
C	-2.223448	2.517219	0.890010
O	-1.957461	1.284358	1.118554
O	-3.304499	3.066790	1.117831

C	-2.323027	-2.651137	0.257993
C	-3.384328	-3.447642	-0.148050
C	-4.491567	-2.840689	-0.722919
C	-4.494475	-1.460033	-0.867950
C	-3.396368	-0.736372	-0.423936
N	-2.331058	-1.318156	0.131936
C	-1.069265	-3.236618	0.854454
O	-0.147137	-2.381368	1.100329
O	-0.988275	-4.450796	1.060144
C	3.426700	-0.719752	0.293197
C	4.651951	-1.235905	-0.104660
C	4.682037	-2.475231	-0.727574
C	3.485534	-3.150669	-0.927443
C	2.305033	-2.568175	-0.487513
N	2.274674	-1.377363	0.114668
C	3.301778	0.635996	0.939038
O	2.096626	1.008770	1.164526
O	4.312237	1.295061	1.199249
U	-0.007648	-0.030823	0.757263
H	-2.178614	5.122484	0.052555
H	-0.231913	6.301275	-1.005568
H	1.896970	5.022420	-1.319727
H	1.999966	2.667149	-0.540903
H	-3.324253	-4.517661	-0.014860
H	-5.334418	-3.432892	-1.054181
H	-5.332397	-0.943032	-1.314672
H	-3.366746	0.339657	-0.505205
H	5.550553	-0.663485	0.071791
H	5.619942	-2.904768	-1.054226
H	3.459006	-4.116165	-1.413313
H	1.355979	-3.067732	-0.611319
O	-0.021147	-0.045379	2.543535

O	-0.006568	-0.023255	-1.028732

Pic$^-$

C	-24.150010	-2.967864	2.991676
C	-24.158280	-2.744014	4.368892
C	-23.423994	-3.577139	5.199961
C	-22.711154	-4.624845	4.632667
C	-22.764384	-4.781788	3.253147
N	-23.455926	-3.975168	2.444552
C	-24.949888	-2.039514	2.063200
O	-24.715430	-2.102395	0.832374
O	-25.779184	-1.280750	2.630576
H	-24.736495	-1.922903	4.764716
H	-23.410333	-3.414117	6.270284
H	-22.127264	-5.305371	5.237800
H	-22.223289	-5.592323	2.776078

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