Electronic Supplementary Information

Chemical reactivity of the tryptophan/acetone/DMSO triad system and its potential applications in nanomaterial synthesis

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Supplementary Methods

1. The HPLC-DAD conditions for the kinetic study

Instrument: Agilent 1260 Infinity II HPLC instrument equipped with a 1260 Infinity II quaternary pump, a 1260 Infinity II vial sampler, a 1260 Infinity II degasser, a 1260 Infinity II column thermostat, a 1260 Infinity II diode array detector, and a PC with the Agilent Chemstation software, all of which were purchased from Agilent Technologies (Waldbronn, Germany).

Column: Mightysil octadecyl silica (ODS) column (5 μm; column of dimensions 4.6 × 250 mm) (Kanto, Tokyo, Japan)

Mobile phase: Gradient separation was conducted with 0.025% formic acid in deionized water (*v/v*) (solvent A) and 0.025% formic acid in methanol (*v/v*) (solvent B) as mobile phase. The gradient condition is shown in the following table.

Temperature: Autosampler (25°C); column oven (25 °C)

Injection volume: 20 μL

Flow rate: 1 mL/min

UV-Vis detection mode: 242, 254, and 280 nm

Mobile phase gradients:

Time (min)	Solvent A (%)	Solvent B (%)
0	85	15
2.5	85	15
17.5	70	30
22.5	70	30
37.5	50	50
42.5	50	50
55	10	90
57.5	10	90
60	85	15
65	85	15

2. The UHPLC-TOF-MS conditions for the identification of mesityl oxide (MO)

Instrument: Agilent 1290 ultra-high-performance liquid chromatography (UHPLC) system (Waldbronn, Germany) with a Bruker maXis ultra-high-resolution (UHR) TOF mass spectrometer (Bruker Daltonics, Bremen, Germany)

Column: Thermo Scientific Accucore aQ C18 Polar Endcapped LC column (2.1 mm × 100 mm, 1.9 μm)

Mobile phase: 10-mM ammonium acetate and 0.1% formic acid in deionized water (solvent A) and 10-mM ammonium acetate and 0.1% formic acid in a 2:3 mixture of methanol and isopropanol (solvent B). The gradient condition is shown in the following table.

Temperature: Autosampler (4°C); column oven (40 °C)

Injection volume: 5 μL

Flow rate: 0.4 mL/min

ESI detection mode: negative mode (dry gas temperature, 200 °C; dry gas flow rate, 8 L/min; capillary voltage, 4500 V). The mass spectra were acquired in a full-scan mode in the range of 50–1500 *m/z*.

Mobile phase gradients:

Time (min)	Solvent A (%)	Solvent B (%)
0	100	0
2	100	0
3	95	5
5	1	99
15	1	99

3. The UPLC-TOF-MS parameters for untargeted profiling

Instrument: Waters ACQUITY ultra-performance liquid chromatography (UPLC) system with a XEVO QTOF mass spectrometer (Milford, MA, USA).

Column: Agilent poroshell 120 EC-C18 column (4.6 mm × 100 mm, 2.7 μm)

Mobile phases: Gradient separation was conducted with 0.1% ammonium hydroxide in deionized water (solvent A) and 0.1% ammonium hydroxide in methanol (solvent B). The gradient condition is shown in following table.

Temperatures: Autosampler (4°C); column oven (45 °C)

Injection volume: 5 μL

Flow rate: 0.5 mL/min

ESI detection mode: negative mode (dry gas temperature, 200 °C; dry gas flow rate, 10 L/min; capillary voltage, 2500 V). The mass spectra were acquired in a full-scan mode in the range of 50–800 *m/z*.

Mobile phase gradients:

Time (min)	Solvent A (%)	Solvent B (%)
0	90	10
5	90	10
20	55	45
60	51.5	48.5
64	10	90
70	10	90
74	90	10
80	90	10

4. The UPLC-TOF-MS parameters for compound confirmation

Instrument: Waters ACQUITY ultra-performance liquid chromatography (UPLC) system with a XEVO QTOF mass spectrometer (Milford, MA, USA).

Column: Agilent poroshell 120 EC-C18 column (4.6 mm × 100 mm, 2.7 μm)

Mobile phases: Gradient separation was conducted with 0.1% ammonium hydroxide in deionized water (solvent A) and 0.1% ammonium hydroxide in methanol (solvent B). The gradient condition is shown in following table.

Temperatures: Autosampler (4°C); column oven (45 °C)

Injection volume: 5 μL

Flow rate: 0.5 mL/min

ESI detection mode: negative mode (dry gas temperature, 200 °C; dry gas flow rate, 10 L/min; capillary voltage, 2500 V). The mass spectra were acquired in a full-scan mode in the range of 50–800 *m/z*.

Mobile phase gradients:

Time (min)	Solvent A (%)	Solvent B (%)
0	99	1
0.5	99	1
4.5	0	100
5	0	100
6	99	1
9	99	1

Supplementary Figures

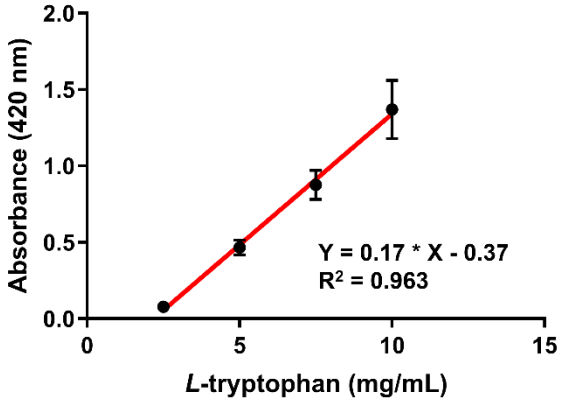

Fig. S1. Linear correlation between *L*-tryptophan concentration and the degree of browning, as indicated by absorbance at 420 nm. The absorbance values were obtained from the spectra data presented in Fig. 1 (absorbance at 420 nm after 1 day browning).

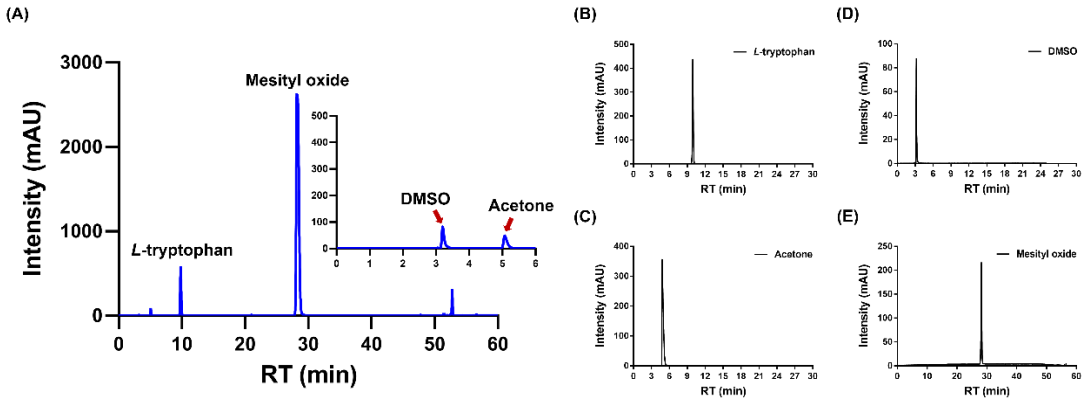

Fig. S2. The chromatograms were acquired for the following substances: (A) *L*-tryptophan reacted in a DMSO and acetone co-solvent system for one day, while reference standards of (B) *L*-tryptophan, (C) acetone, (D) DMSO, and (E) mesityl oxide were utilized to obtain their respective chromatograms. Peaks were identified based on their retention times (RT).

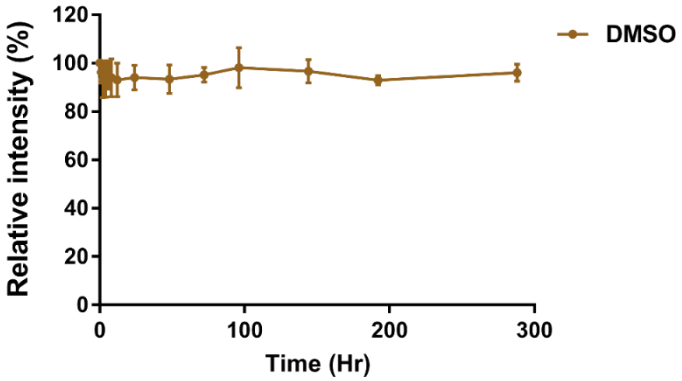

Fig. S3. The quantity of DMSO (indicated by relative peak intensity detected at 242 nm in HPLC) remained constant throughout the 12-day browning reaction. All data are presented as means ± SD.

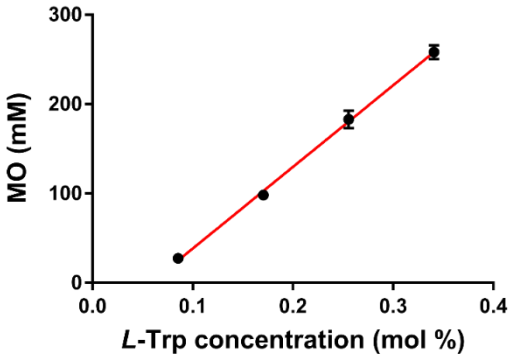

Fig. S4. The correlation between *L*-tryptophan concentration (mol %) and the quantity of mesityl oxide (MO) generated within 24 hours in the closed reaction system. Data are presented as means ± SD (n = 3).

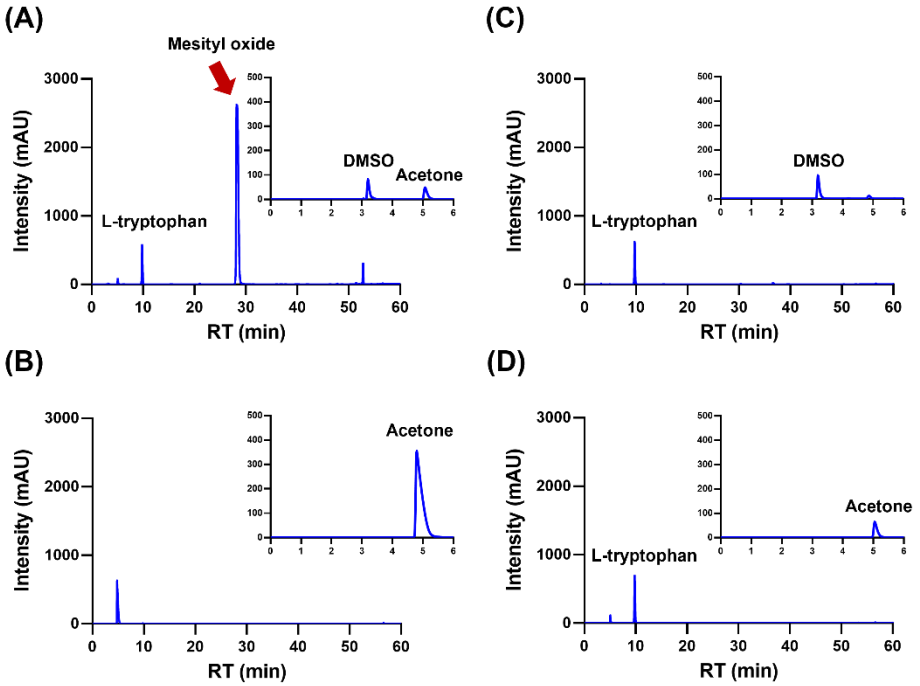

Fig. S5. The chromatograms were acquired for different reaction conditions. (A) *L*-tryptophan reacted in a DMSO and acetone co-solvent system. (B) *L*-tryptophan reacted in acetone alone; note that *L*-tryptophan is not soluble in acetone. (C) *L*-tryptophan reacted in DMSO alone. (D) *L*-tryptophan reacted in a ddH_2O and acetone co-solvent system for one day. Peaks were identified based on their retention times (RT).

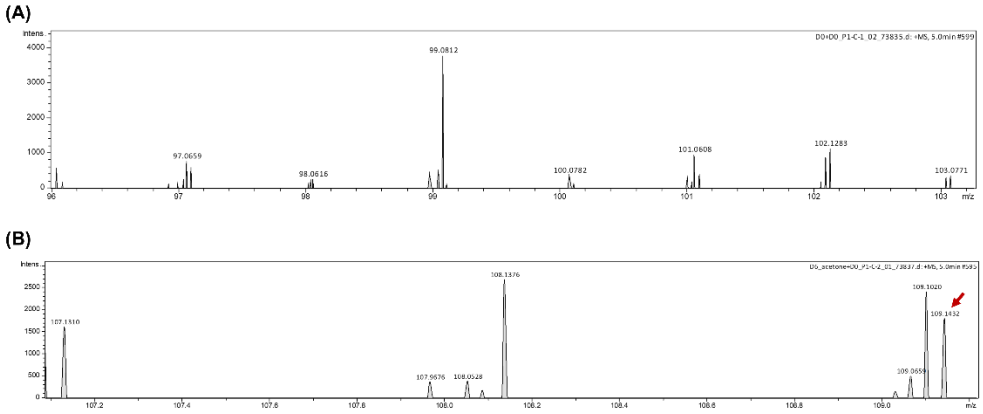

Fig. S6. High resolution mass spectrometry identification of mesityl oxide ($C_6H_{10}O$) in an *L*-tryptophan browning solution within 3 hours. The compound was detected under two conditions: (A) Using acetone-d0 as the acetone source, the theoretical $[M+H]^+$ m/z was 99.0809, with a mass error of 3.02 ppm. (B) When acetone-d6 was used as the acetone source, the theoretical $[M+H]^+$ m/z was 109.1432, with a mass error of 4.58 ppm.

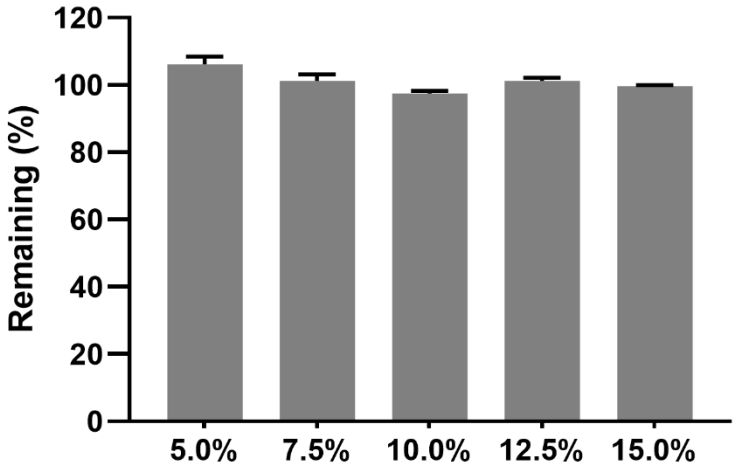

Fig. S7. Remaining percentage of DMSO in the DMSO/acetone/*L*-tryptophan reaction system after 3 days at room temperature. Data presented as mean ± SD, n = 3.

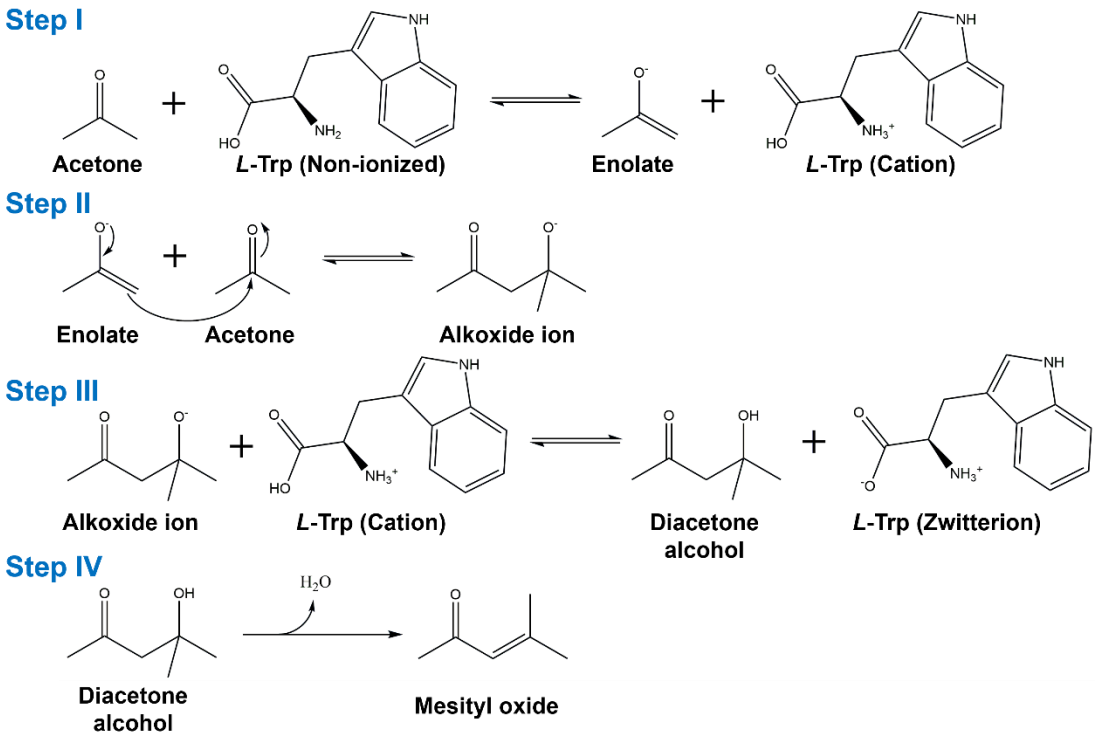

Fig. S8. Plausible mechanism of self-aldol condensation of two acetone molecules in DMSO with the assistance of *L*-tryptophan.

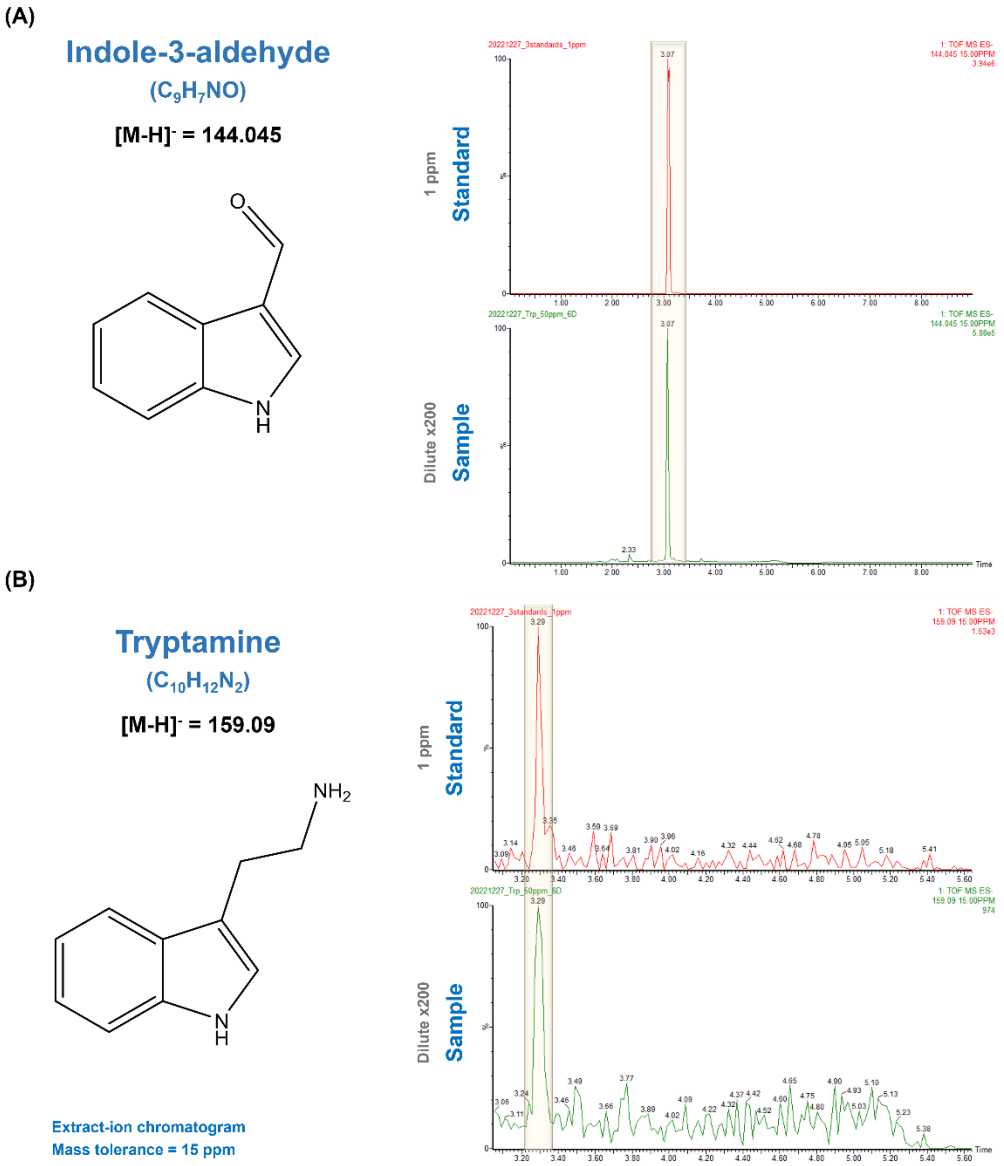

Fig. S9. Comparison of extracted ion chromatograms was performed between a 1 μg/mL standard solution and a diluted sample solution to assess the presence of (A) indole-3-aldehyde and (B) tryptamine. The analyzed sample was the *L*-tryptophan browning solution following a reaction period of 6 days.

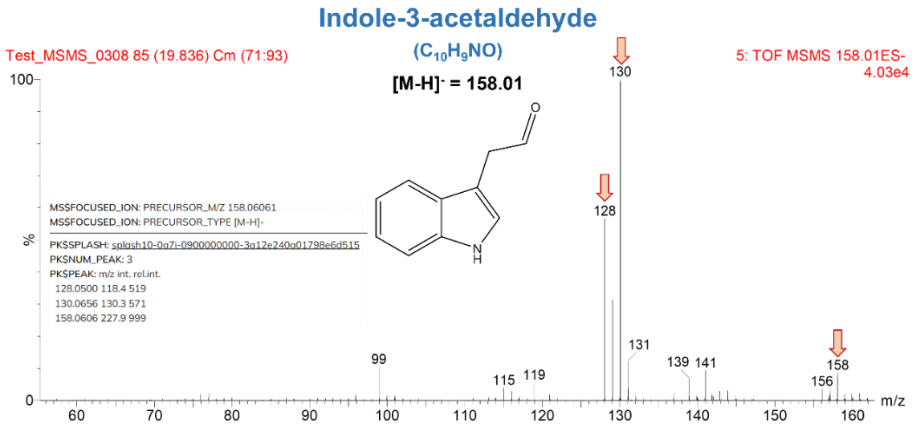

Fig. S10. LC-MS/MS spectrum of indole-3-acetaldehyde (identification via database comparison).

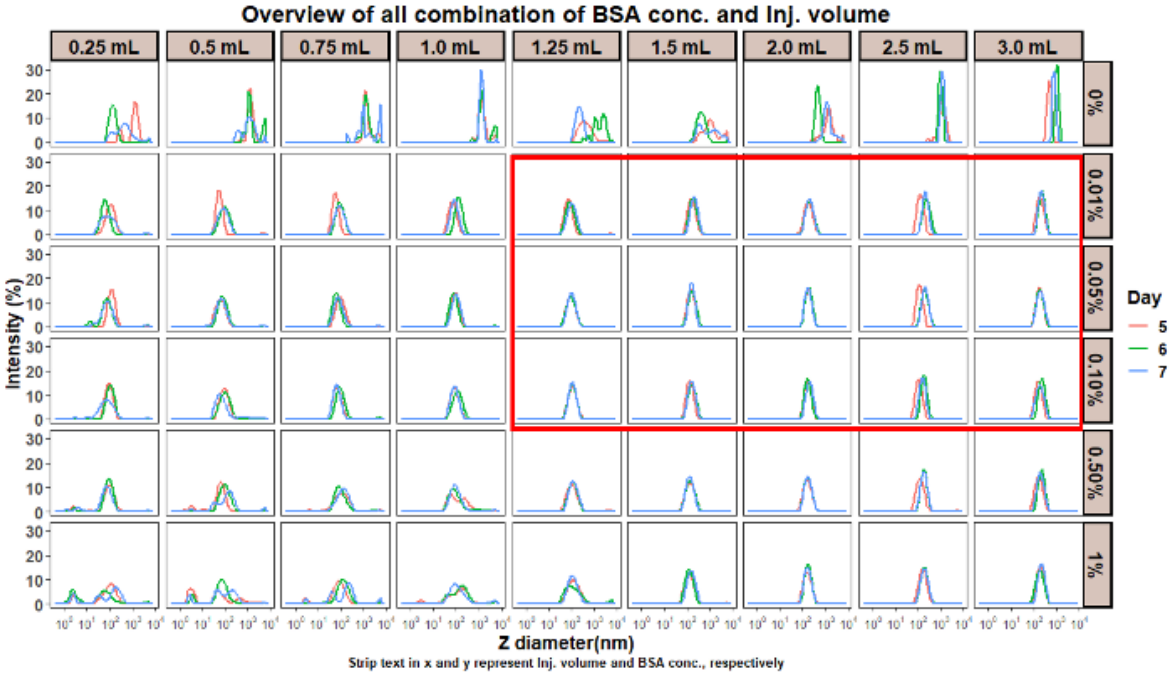

Fig. S11. Optimization of nanoparticle preparation via solvent-mediated browning of *L*-tryptophan. The organic phase comprises *L*-tryptophan (10 mg/mL) dissolved in a mixture of DMSO/acetone (90% v/v/10% v/v). After undergoing browning for 5, 6, or 7 days, different quantities of the organic phase were introduced into 10 mL of water with varying concentrations of bovine serum albumin (BSA). Size distribution plots are organized and displayed in a matrix format, with columns representing the volume of the organic phase injected into 10 mL of water, and rows representing the concentration of bovine serum albumin in the aqueous phase. Conditions resulting in stable and uniform nanoparticles (with a polydispersity index, PDI, ranging from 0.08 to 0.17) are highlighted within the red frame.

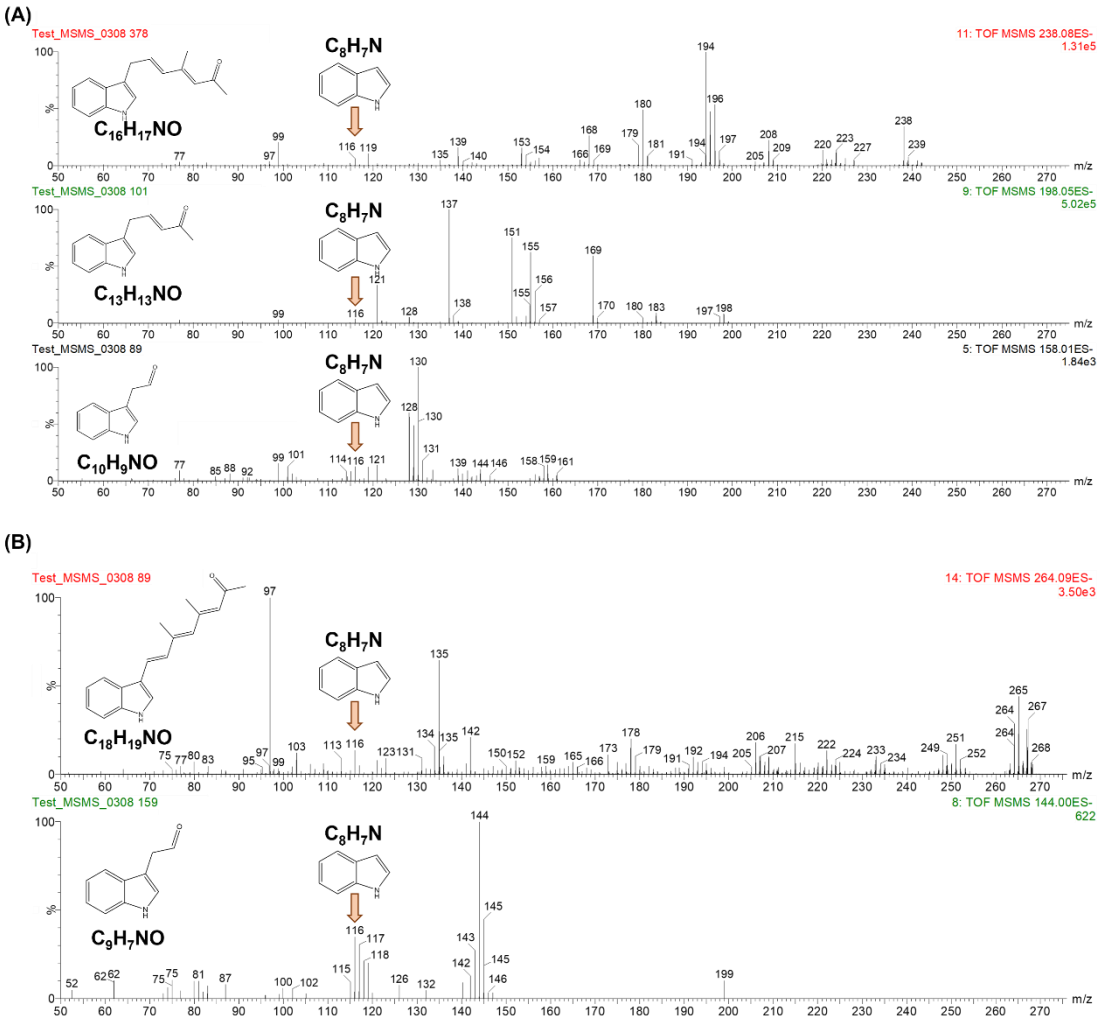

Fig. S12. Representative UPLC-TOF-MS/MS mass spectra for tentative indole-containing molecules extracted from nanoparticles. (A) $C_{10}H_9NO$, $C_{13}H_{13}NO$, $C_{16}H_{17}NO$; (B) C_9H_7NO, $C_{18}H_{19}NO$. Methanol was used to dissolve the nanoparticles.

Supplementary Tables

Table S1. Retention time, regression analysis, LODs, and LOQs for *L*-Tryptophan (L-Trp), acetone, and mesityl oxide (MO) in HPLC-DAD analysis.

Compounds	t_m (min)[a]	Dilute solvent	Regression equation, $Y = a + bX$[b]		Correlation coefficient	LOD[a]	LOQ[a]
			a	*b*			
L-Trp (280 nm)	9.9	ddH_2O	66.994	29.879	0.9998	172.977	524.173
Acetone (254 nm)	4.9	ddH_2O	8.0383	165.3	0.9911	1.844	5.587
MO (242 nm)	28.3	ddH_2O	-37.622	82.192	0.9998	0.389	1.46
MO (280 nm)	28.3	ddH_2O	1.3484	8.8224	0.9999	9.601	29.094
MO (242 nm)	28.1	DMSO	61.393	141.22	0.9999	0.371	1.125
MO (280 nm)	28.1	DMSO	23.024	2.290	0.9999	9.824	29.771

[a] t_m: Retention time; LOD: Limit of detection; LOQ: Limit of quantification.

[b] Y refers to the peak area, X is the concentration of compounds (μg/mL for *L*-Trp and MO; μL/mL for acetone).

Table S2. Extracted equivalent masses (*m/z*) from the browning reaction system of *L*-tryptophan in a DMSO-acetone mixture (9:1) for 6 days. The tentatively identified molecular formulas, adduct type, retention time (RT), experimental *m/z*, theoretical *m/z*, and mass errors (in ppm) of these features are presented.

Tentative identified molecular formula	Adduct type	RT (min)	Exp. *m/z*	Theor. *m/z*	Mass error (ppm)
C_9H_7NO	$[M-H]^-$	18.005	144.0451	144.0449	1.32
$C_{10}H_9NO$	$[M-H]^-$	16.529	158.0605	158.0606	-0.54
$C_{11}H_9NO$	$[M-H]^-$	23.216	170.0602	170.0606	-2.07
$C_{10}H_{10}N_2O$	$[M-H]^-$	14.075	173.0712	173.0715	-1.95
$C_{12}H_{11}NO$	$[M-H]^-$	26.082	184.0763	184.0762	0.4
$C_{13}H_{13}NO$	$[M-H]^-$	27.593	198.0921	198.0919	0.85
$C_{11}H_{12}N_2O_2$	$[M-H]^-$	19.916	203.0817	203.0821	-1.81
$C_{14}H_{13}NO$	$[M-H]^-$	37.695	210.0918	210.0919	-0.68
$C_{13}H_{11}NO_2$	$[M-H]^-$	30.762	212.0711	212.0712	-0.54
$C_{13}H_{13}NO_2$	$[M-H]^-$	21.472	214.0870	214.0868	0.76
$C_{15}H_{15}NO$	$[M-H]^-$	63.644	224.1088	224.1075	5.62
$C_{16}H_{17}NO$	$[M-H]^-$	55.566	238.1230	238.1232	-0.78

$C_{17}H_{17}NO$	$[M-H]^-$	65.171	250.1241	250.1232	3.77
$C_{16}H_{17}NO_2$	$[M-H]^-$	33.714	254.1182	254.1181	0.43
$C_{18}H_{19}NO$	$[M-H]^-$	64.376	264.1383	264.1388	-1.72
$C_{19}H_{21}NO$	$[M-H]^-$	64.971	278.1559	278.1545	4.95
$C_{19}H_{24}N_2$	$[M-H]^-$	19.166	279.1865	279.1861	1.4
$C_{20}H_{21}NO$	$[M-H]^-$	65.669	290.1553	290.1545	2.86
$C_{19}H_{20}N_2O$	$[M-H]^-$	64.256	291.1498	291.1497	0.46
$C_{19}H_{23}NO_2$	$[M-H]^-$	38.525	296.1645	296.1651	-2.09
$C_{19}H_{26}N_2O$	$[M-H]^-$	64.874	297.1964	297.1967	-1.13
$C_{22}H_{28}N_2$	$[M-H]^-$	68.569	319.2170	319.2174	-1.13
$C_{21}H_{26}N_2O$	$[M-H]^-$	65.600	321.1970	321.1967	1.02
$C_{22}H_{27}NO_2$	$[M-H]^-$	65.286	336.1962	336.1964	-0.46
$C_{22}H_{30}N_2O$	$[M-H]^-$	65.732	337.2256	337.228	-7.1
$C_{22}H_{28}N_2O_2$	$[M-H]^-$	65.435	351.2016	351.2073	-16.1
$C_{21}H_{19}N_3O_3$	$[M-H]^-$	14.258	360.1339	360.1348	-2.52
$C_{25}H_{34}N_2O$	$[M-H]^-$	24.423	377.2587	377.2584	0.86
$C_{25}H_{26}N_4O_3$	$[M-H]^-$	15.299	429.1916	429.1927	-2.65
$C_{26}H_{45}N_5O_5$	$[M-H]^-$	69.519	506.3336	506.3342	-1.18