Electronic Supplementary Material (ESI) for Chemical Communications

A rapid and highly selective colorimetric method for direct detection of tryptophan in proteins via DMSO acceleration

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Chemicals and Apparatus: All 20 standard amino acids including L-tryptophan were purchased from Sigma. Hydrochloric acid (37%) and formic acid (88%) were from Beijing Chemical Plants. Dimethyl sulfoxide (DMSO), *N*,*N*'-dimethylformamide (DMF) and ethanol were of analytical grade and also from Beijing Chemical Plants. Acetonitrile and methanol (HPLC grade) was from Fisher Scientific. Ultra-pure water from a MilliQ water purification system (Millipore, Bedford, MA) was used throughout. Amino acid, peptide and protein solutions were prepared in water. Indole-3-propanic acid (IPA) solution was prepared in ethanol.

The absorption spectra were measured with a UV-1800 UV spectrophotometer (Shimadzu, Japan). HPLC and LC-MS analysis were performed on a SUPELCO C_{18} (4.6×150 mm) column. A Shimadzu Prominence UFLC system combined with photo-diode array detector (Japan) and a Shimadzu LCMS-2010 (Japan) were used for the characterization of the reaction solutions. Accurate molecular weight and elemental composition of the products were identified by an APEXII fourier transform ion cyclotron resonance mass spectrometry (FTICR MS) (Bruker Daltonics Inc.).

General Experimental: a typical reaction solution was prepared by adding tryptophan (25 mM, 1.0 mL) to a mixture containing concentrated hydrochloric acid (37%, w/w, 2.5 mL), formic acid (88%, w/w, 1.0 mL) and 0.2% (v/v) DMSO. The final volume of this solution was diluted to 5.0 mL with water. After reacting at 50 ^{o}C or room temperature for different time, the reaction solutions were analyzed by either UV spectrophotometer or HPLC.

The absorption spectra were measured in 1-cm quartz cells. Reaction blank (6M HCl and 18% formic acid) treated under the same condition was used as the reference. The HPLC was performed on a SUPELCO C_{18} (4.6×150 mm) column and the condition for the separation of the reaction solution was chosen as 0-10-10.1-30-35 min, 7%-7%-20%-80%-80% acetonitrile containing 0.1% TFA. The flow rate was 1.0 mL/min. When using photo-diode array detector, the scan wavelength was set at the range of 200 – 800 nm.

Specificity of the catalytic effect of DMSO on the colorimetric reaction. Different organic solvents were tested for their influence on the reaction of tryptophan in aqueous solution containing 18% formic acid and 6 M HCl. After 90 min, the mixed solutions added with acetonitrile, methanol, ethanol or DMF were colorless, while DMSO added solution showed distinct violet blue color (Figure S1, insert). The results were also demonstrated by the measurement of their absorption spectra (Figure S1).

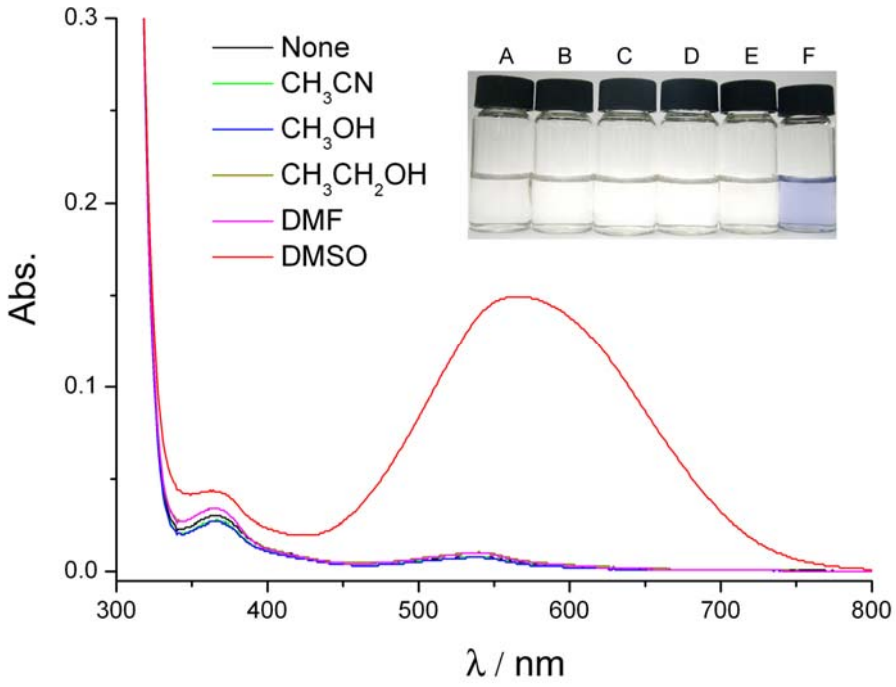

Figure S1. Absorption spectra of tryptophan after treatment at 50 ^{o}C for 90 min in an aqueous solution containing 18% formic acid, 6 M HCl and different additives (0.2%). The inset shows the photograph of tryptophan treated with mixed solutions of 18% formic acid and 6 M HCl (A) added with CH_3CN (B), CH_3OH (C), CH_3CH_2OH (D), DMF (E) or DMSO (F) after heated for 90 min, respectively.

The specificity of the rapid colorimetric reaction of tryptophan. To verify the specificity of the present method, contrast experiments were carried out towards 20 standard L-amino acids (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine). After treated under the same conditions by 6 M HCl, 18% formic acid and 0.2% DMSO, all these amino acids showed no color reaction in the mixed solution except tryptophan (Figure S2). The results demonstrated the rapid colorimetric method can be used for the highly specific detection of tryptophan without the interference of other 19 natural amino acids.

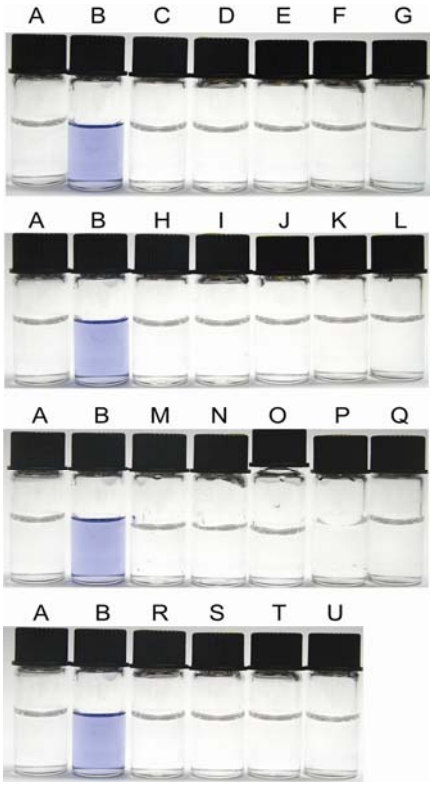

Figure S2. Color reactions of amino acids in 6M HCl, 18% formic acid and 0.2% DMSO. A) Reagent blank (6 M HCl, 18% formic acid and 0.2% DMSO); B) L-tryptophan; C) L-glutamic acid; D) L-glutamine; E) L-glycine; F) L-histidine; G) L-isoleucine; H) L-leucine; I) L-lysine; J) L-methionine; K) L-phenylalanine; L) L-proline; M) L-alanine; N) L-aspartic acid; O) L-asparagine; P) L-arginine; Q) L-cysteine; R) L-serine; S) L-threonine; T) L-tyrosine; U) L-valine. The concentration of each amino acid was 10 mM. The color reactions were carried out at 50 °C for 5 h.

Figure S3. Colorimetric reactions of different compounds in HCl/HCOOH with 0.2% DMSO (left) and without DMSO (right). The treatment was carried out at 50 ^{o}C for 180 min (gramicidin), 120 min (trypsin), 150 min (lysozyme) or 10 min (indole-3-acetic acid), respectively.

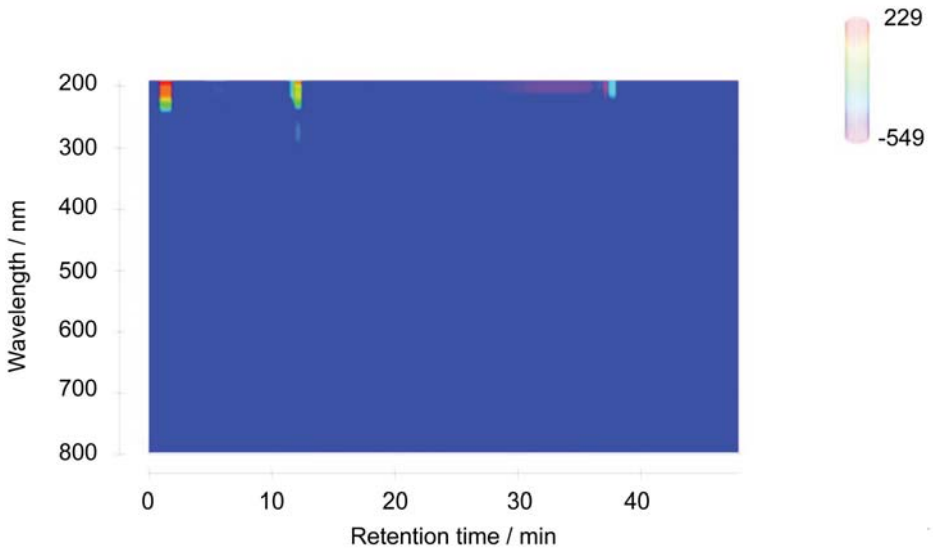

Figure S4. Chromatographic separation of the reaction mixtures monitored by photo-diode array detector at the range of 200 – 800 nm. The reaction mixtures were prepared by treating tryptophan with 6 M HCl, 18% formic acid and 0.2% DMSO.

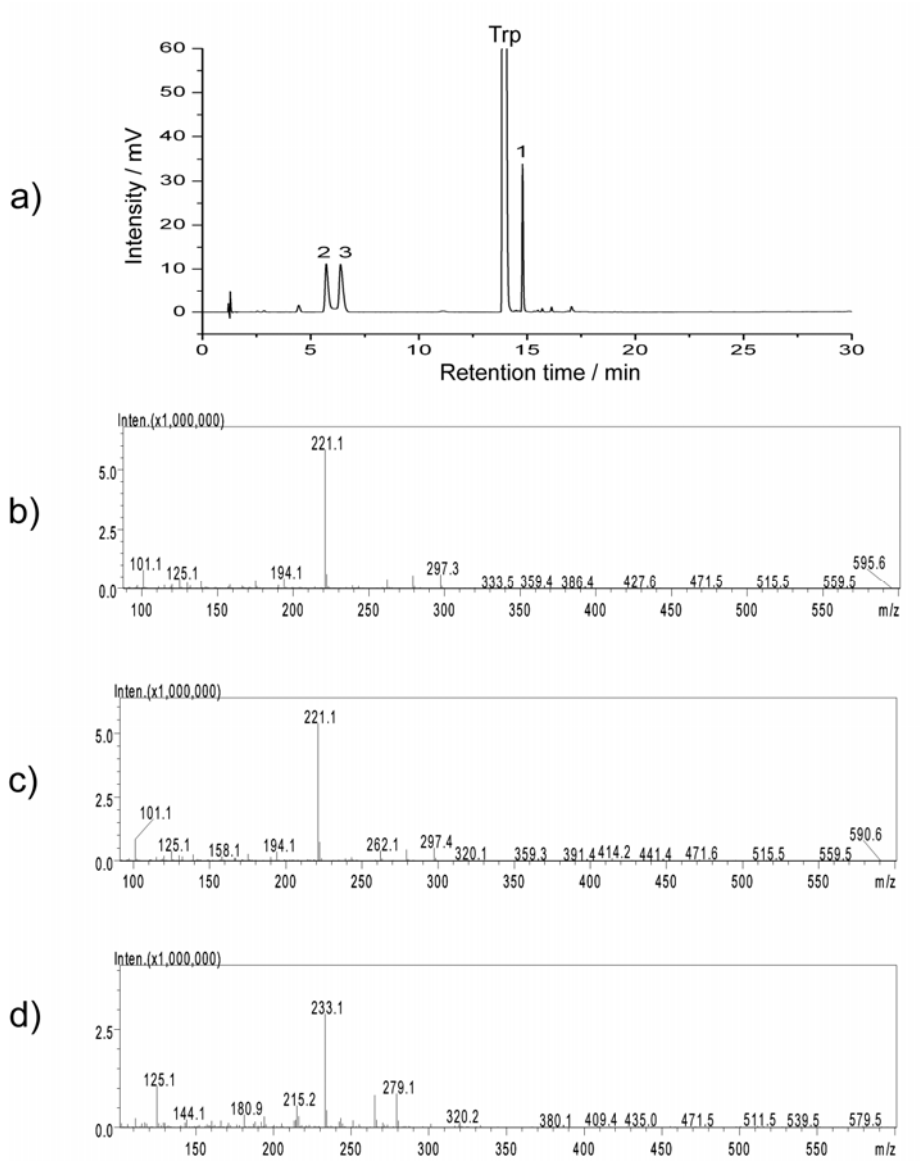

Figure S5. LC-MS analysis of the colorimetric reaction mixture (5 mM tryptophan treated with 6 M HCl, 18% formic acid and 0.2% DMSO at 50 ^{o}C for 5 h). (a) Chromatogram for the separation of reaction mixture. (b – d) Positive ESI mass spectra of peak 2 (retention time: 5.51 min) (b), peak 3 (retention time: 6.15 min) (c) and peak 1 (retention time: 14.77 min) (d).

Determination of the oxidation products as oxindolylalanine (Oia) by HPLC. Oxindolylalanine (Oia) was prepared according to the literature,[1] and then was separated by HPLC. Under the same conditions, two peaks were separated for Oia (Figure S6, lower panel) which showed identical retention time and peak shape with peak 2 and 3 separated from the colorimetric reaction mixture of tryptophan (Figure S6, upper panel). Together with the fact that the species in peak 2 and 3 have the same molecular weight and elemental composition with Oia, these two peaks can be assigned as the keto species and the enol species of Oia, which exist in equilibrium. The inserted photographs show that the brown-colored Oia solution did not contribute

to the production of blue color in the colorimetric reaction of tryptophan.

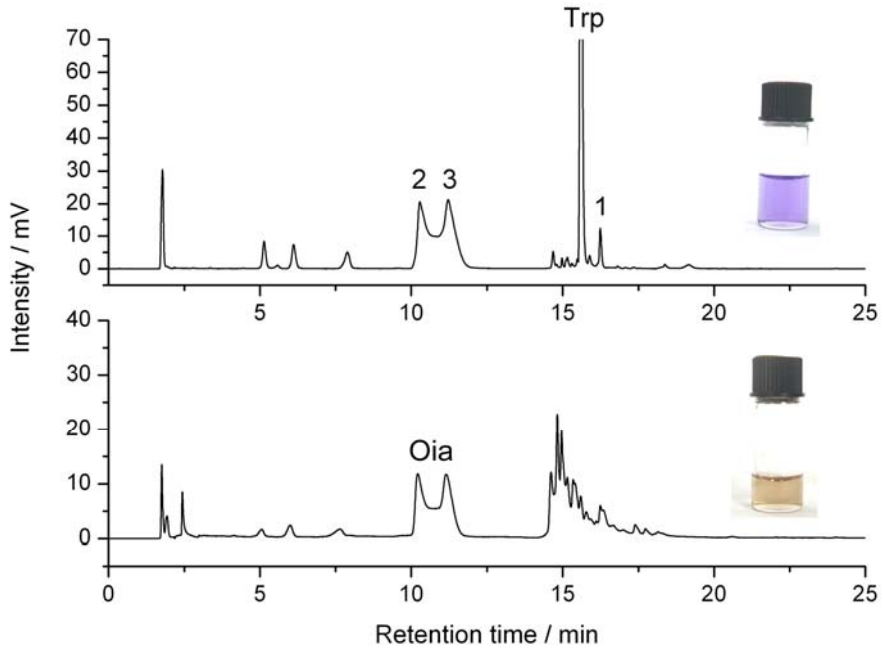

Figure S6. HPLC separation of different reaction solutions of tryptophan. The colorimetric reaction was carried out by treating tryptophan with 6 M HCl, 18% formic acid and 0.2% DMSO at 50 ^{o}C for 5 h (upper panel). The oxidation reaction resulting in Oia was carried out by treating tryptophan with DMSO-HCl-CH_3COOH 1 : 5 : 5 (v/v). The insert shows the photographs of the resultant reaction solutions.

By using photo-diode array detector, the UV-Vis spectra of peak 2 and peak 3 separated from the HCOOH/HCl/DMSO system were scanned (Figure S7). Both species gave a peak at 250 nm which was a representative absorbance of Oia.[2] No absorption band at the visible region was observed for these peak species, further confirming that they had no relationship with violet blue pigment.

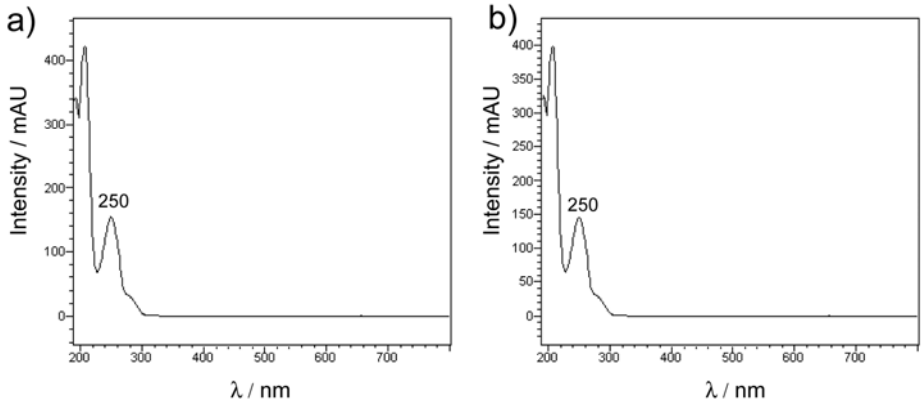

Figure S7. UV-Vis spectra of peak 2 (a) and peak 3 (b) separated from the colorimetric reaction solution of tryptophan.

Stability of the violet blue product with varied pH values. After tryptophan reacted in the aqueous solution of 6 M HCl, 18% formic acid and 0.2% DMSO at 50 ^{o}C for 90 min, the pH value of the mixture was adjusted with NaOH or HCl to examine the stability of the resultant blue pigment. As shown in Figure S8, the maximum absorption at about 580 nm decreased as the pH increased. The absorbance nearly reached baseline when the pH value was 6. More interestingly, the blue color could be rapidly restored when the reaction solution was acidified back to strong acid at room temperature. Although such acid-base equilibrium was also found in the none-DMSO reaction system, the recovery of the blue color in the none-DMSO system only could be achieved by incubating at 50 ^{o}C overnight.[3] The results demonstrated the acid-dependent stability of the blue pigment. And the addition of DMSO also significantly accelerated the recovery of the violet blue color from the acid-base equilibrium.

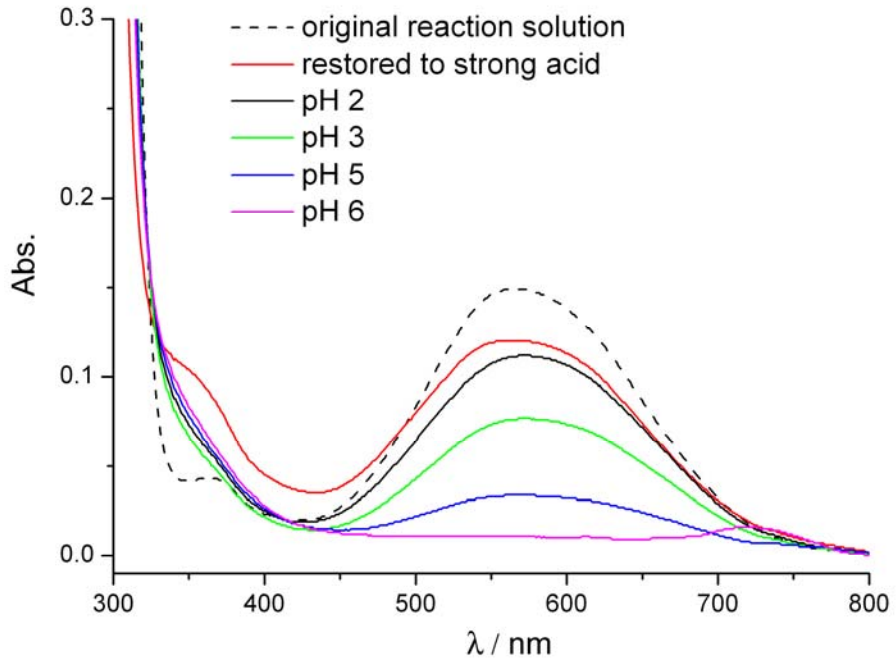

Figure S8. Spectral changes of reaction solutions of tryptophan (5 mM) after adjusted with NaOH to different pH values: original reaction solution (dash line); pH 2 (black); pH 3 (green); pH 5 (blue); pH 6 (purple); restored with concentrated HCl to strong acid (red). The original reaction solution was achieved by treating tryptophan at 50 °C for 90 min in HCOOH/HCl/DMSO.

Discussion on the effect of DMSO on the production of the colored derivative. Based on the fact that DMSO can distinctly accelerate the colorimetric reaction, further experiments were carried out to investigate which step of the colorimetric reaction was more affected from the addition of DMSO. As shown in Figure S9a, the absorption band at visible region increases with the increasing amount of DMSO after treating at 50 ^{o}C for 45 min. Meanwhile, those reaction solutions were analyzed by

HPLC, respectively (Figure S9b). Due to the instability and unavailability of the final blue derivative, the formation and consumption of its precursor 1-formyltryptophan can give some insight into the catalytic mechanism of DMSO. The areas of peak 1 which represented the amount of 1-formyltryptophan decreased as the content of DMSO increased. The results conferred that the consumption rate of 1-formyltryptophan was more elevated by DMSO, which resulted in the improved generation of blue pigment.

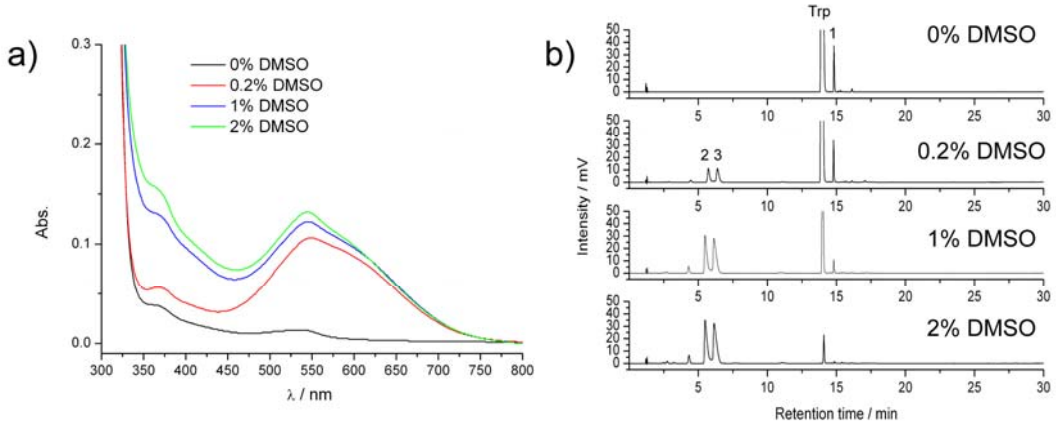

Figure S9. Absorption spectra and HPLC chromatograms of colorimetric reaction solutions. The reactions were carried out by treating tryptophan with 6 M HCl, 18% formic acid and different amount of DMSO at 50 ^{o}C for 45 min.

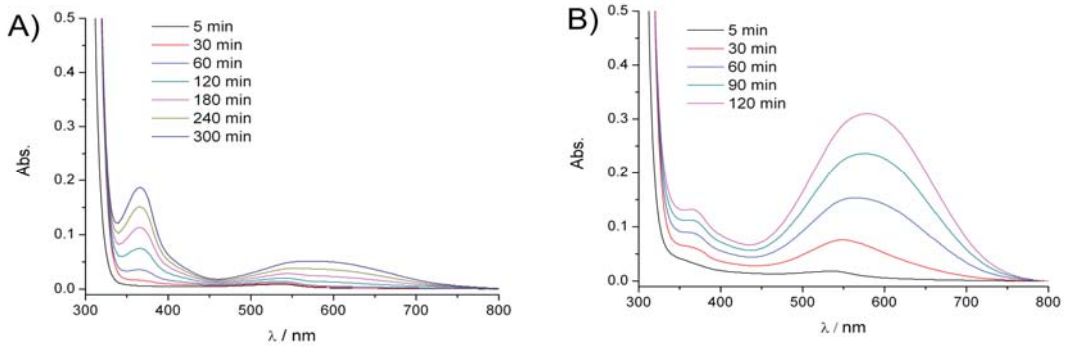

Figure S10. Absorption spectra of Trp (10 mM) in solutions containing 6 M HCl and 18% formic acid with 0% (A) and 0.2% DMSO (B). The reactions were performed at 50 ^{o}C for different times.

References

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2. J. W. Cornforth, C. E. Dalgliesh and A. Neuberger. *Biochem. J.* 1951, **48,** 598.
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