Regioselective inverse Diels-Alder reaction of unsymmetrical tetrazines with aldehydes and ketones

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Dedicated to Professor Manfred Schlosser in honor of his scientific achievements

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Abstract
The amine-catalyzed inverse electron demand Diels-Alder reaction of unsymmetrical tetrazines with aldehydes and ketones was investigated. We found that only one regioisomer could be observed when pyrrolidine was applied as the catalyst. Using this reaction, fluorogenic dyes could be developed with a tetrazine unit as the “triggering group”.

Keywords: Tetrazine, Diels-Alder reaction, regioselectivity, enamine, pyridazine

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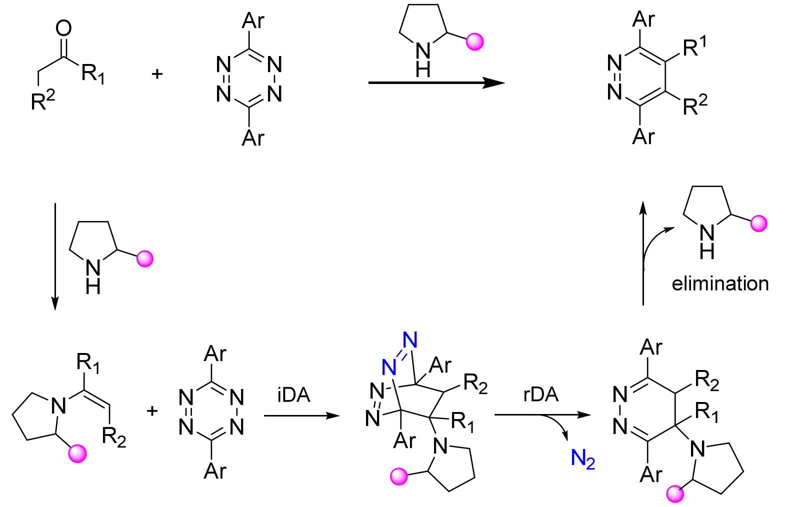
Introduction

Tetrazines are useful reagents that can react with electron-rich dienophiles to produce pyridazines.[1-4] The reaction goes through two steps: an inverse-electron-demand Diels-Alder reaction (iDA) and a retro Diels-Alder (rDA) reaction, which results in the loss of a nitrogen molecule to produce pyridazine as the final product.[5] Due to the high efficiency of this reaction under mild conditions, it can be considered as a type of “Click Chemistry”,[6-9] and recently has been widely used in the synthesis of heterocyclic compounds,[10] in bioconjugation applications,[11-12] as well as in the modulation of fluorescence of dyes for bioimaging applications.[13-14] In addition, substituted pyridazines can be used as ligands for the self-assembly of [2 × 2] grid-like metal complexes with copper (I) and silver (I) ions.[16-19]

To perform the reaction under mild conditions, strained or electron-rich dienophiles are needed to react with tetrazines.[20] On the other hand, although enamines and enolates are two major types of electron rich dienophiles, their low stability greatly compromises their potential applications in organic synthesis. To circumvent this, Wang and coworkers used proline as a catalyst to promote direct iDA reactions of ketones with tetrazines via the *in situ* formation of the enamine intermediate (Scheme 1).[21] However, only symmetric tetrazines were tested in that study. In this paper, we report the regioselectivity of the direct iDA reaction of aldehydes and ketones with unsymmetrical tetrazines as well as the potential application of this protocol in bioimaging.

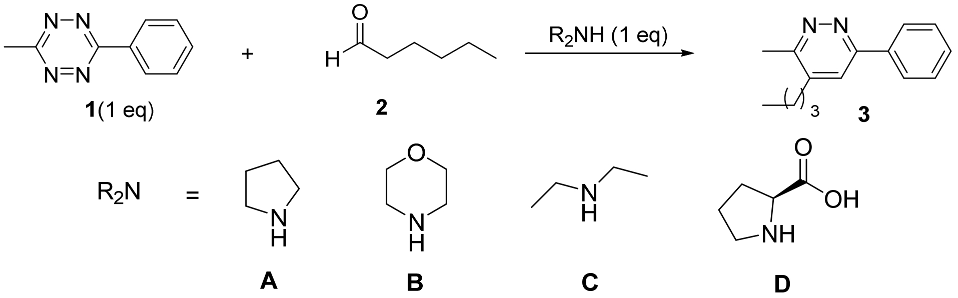


Scheme 1. Mechanism of secondary amine-catalyzed iDA reaction between aldehydes and tetrazines.

Results and Discussion

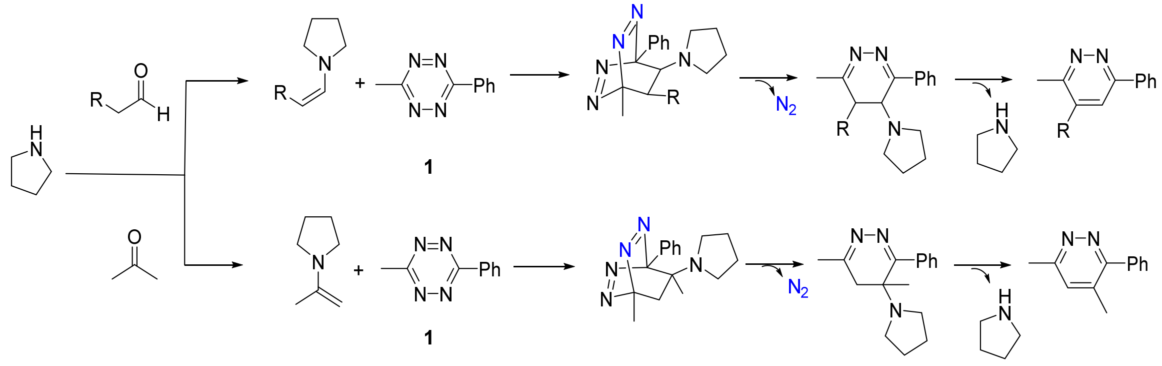
To investigate the reactivity and regioselectivity of the secondary amine-catalyzed iDA reaction of unsymmetrical tetrazines with aldehydes and ketones, a selection of secondary amines (A-D) was screened as catalysts for the reaction of 3-methyl-6-phenyl-1,2,4,5-tetrazine (**1**) with hexanal (**2**). All reactions proceeded smoothly at room temperature to produce a single cycloadduct **3**, in 45-95% yield. The use of pyrrolidine and morpholine in acetonitrile or dichloromethane furnished the product in 89-95% yield within 5 minutes (Entries 1, 2, 4); whereas using diethylamine took 15 minutes (Entry 5). When we replaced the secondary amines with proline, similar yields were observed in DMSO (Entry 9-10), but no products were observed when other solvents were used, even with heating. The use of acetonitrile and water (1:1) resulted in a 45% yield (Entry 3), and a significant amount of unidentified by-products. As control reactions, by mixing the tetrazine with secondary amines in the absence of aldehydes or ketones, we observed no product formation, but a gradual degradation of the starting material over 24 hours. This also could be confirmed by NMR, and by observing the disappearance of the purple color of the tetrazine.

Table 1. Regioselectivity of secondary amine-catalyzed iDA reaction of hexanaldehyde with an unsymmetrical tetrazine



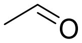
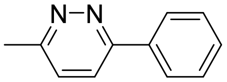
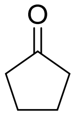
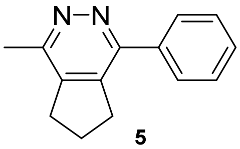
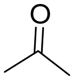
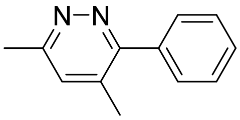
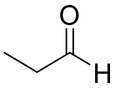
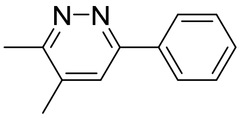
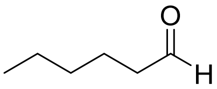
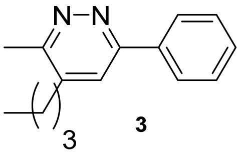
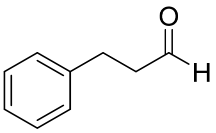
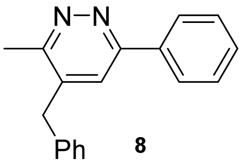
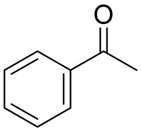
Entry	R_2NH	Solvent	Temperature	Time	Yield (%)
1	A	CH_2Cl_2	rt	5 min	95
2	A	CH_3CN	rt	5 min	89
3	A	$CH_3CN:H_2O$ (1:1)	100 °C	24 h	45
4	B	CH_2Cl_2	rt	5 min	95
5	C	CH_2Cl_2	rt	15 min	94
6	D	CH_2Cl_2	rt	24 h	0
7	D	CH_3CN	rt	24 h	0
8	D	toluene	100 °C	24 h	0
9	D	DMSO	rt	24 h	94
10	D	DMSO	100 °C	10 min	95

Using pyrrolidine as the catalyst, we studied the reactivity and regioselectivity of a number of aldehydes and ketones with the same asymmetric tetrazine 1 (Table 2). NMR analysis of the crude products taken after each reaction unambiguously confirmed the formation of a sole isomeric product in each case (Table 2). Upon purification, single crystal analysis of some of the products were obtained confirm their regioselectivity. Figure 1 shows the crystal structure of the product obtained by the reaction of the tetrazine with 3-phenylpropionaldehyde (Table 2, Entry 6). The regioselectivity of the products formed was consistent with what one would expect according to the zwitterionic models of alignment of dienophiles with unsymmetrical dienes during the Diels-Alder reaction.[22] In this case, the transition state of the intermediate favors placement of the secondary amine and the phenyl substituent of the tetrazine on the same side (Scheme 2).



Scheme 2. Regioselective reaction with unsymmetrical tetrazine.

Table 2. Pyrrolidine-catalyzed regioselective reaction of 3-methyl-6-phenyl-1,2,4,5-tetrazine with aldehydes/ketones

Entry	Aldehyde/Ketone	Product	Yield(%)
1		 **4**	99
2		 **5**	99
3		 **6**	94
4		 **7**	98
5		 **3**	95
6		 **8**	89
7		No reaction	ND

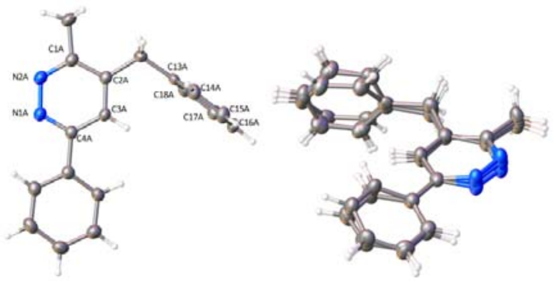
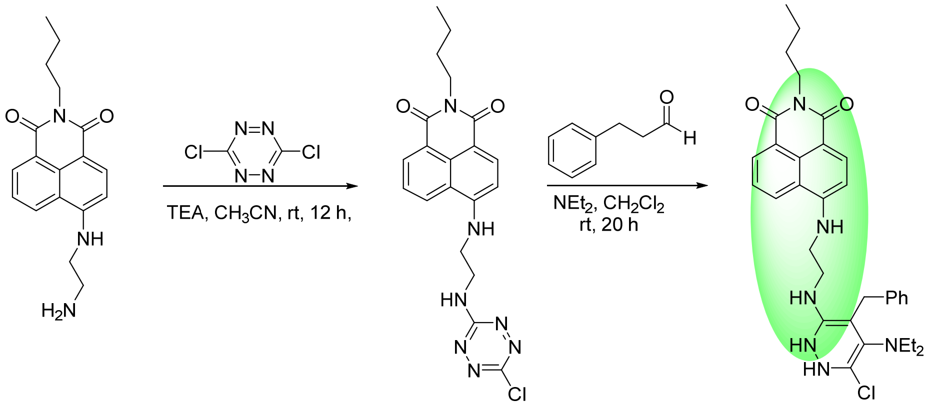


Figure 1. Crystal structure of the 4-ethyl-3-methyl-6-phenylpyridazine (Entry 6 in Table 2).

We further explored the possibility of using this reaction to modulate the fluorescence of dyes by making a fluorogenic naphthalimide-tetrazine dye. As shown in Scheme 3, the dye was prepared by addition of dichlorotetrazine to the amine terminal of **9** to obtain **10**, which subsequently underwent diethylamine-catalyzed iDA reaction with 3-phenylpropionadehyde to furnish a non-aromatized adduct **11**. The elimation of the amine to produce pyradizine was not observed in this instant, indicating the relative stability of the dihydropyridazine of compound **11** over those of the analogues tetrazines in Table 2. Compound **10** was almost completely non-fluorescent due to the quenching effect of the tetrazine motif. However, upon an iDA reaction with an aldehydes or a ketone, e.g. phenylpropionaldehyde, a highly fluoresced **11** could be produced. Photophysical studies of the dyes showed that relative to the quenched fluorophore **10**, the turn-on ratio after reacting with the aldehyde is 635 fold in dichloromethane (Table 3). This is a considerable improvement over reported fluorophore-tetrazine pairs joined by a flexible linker, which yielded turn-on ratios of only 15-20 folds after reaction with dienophiles.[11,13,23] The only reported fluorophore-tetrazine pairs with turn-on ratios greater than we report here had tetrazine directly linked to phenyl rings appended to the fluorophores.[14,24]



9

10 (weak fluorescence)

11 (high fluorescence)

Scheme 3. Synthesis of fluorogenic naphthalimide-tetrazine dye.

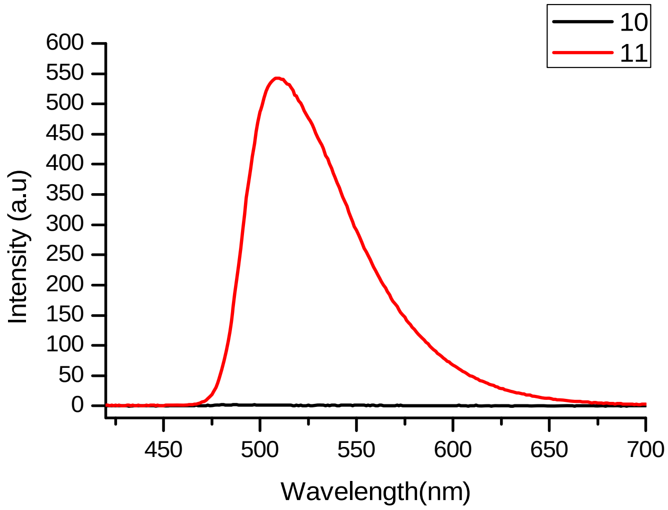


Figure 2. Emission spectra of NT-tetrazine 10 (10 µM) and the corresponding iDA product, NT-diazine 11 (10 µM) in CH_2Cl_2.

Table 3. Photophysical properties of the NT-tetrazine before and after iDA reaction with propionaldehyde

Compound	λ_{abs}	λ_{em}	Quantum Yield
NT-tetrazine (10)	428	507	8.0×10^{-4}
NT-diazine(11)	435	507	0.51

Conclusions

We have established the regioselectivity of a secondary amine-catalyzed iDA reaction with unsymmetrical tetrazines such as 3-methyl-6-phenyl-1,2,4,5-tetrazine, and applied this unique reaction to tuning fluorogenic dyes having a tetrazine motif. We envisage that this method will find utility in imaging of the intracellular distribution of target molecules. Investigations are therefore ongoing to apply this method to target small molecules inside living cells.

Experimental Section

General. 3-Methyl-6-phenyl-1,2,4,5-tetrazine **1**,[25] naphthalimide dye **9**,[26] and dichlorotetrazine[27] were prepared according to literature procedures. All other reagents and solvents were of commercial grade. NMR spectra were recorded on Bruker 300 or 400 instruments. Quantum yield measurements are in triplicate with Rhodamine 6G (in EtOH) as standards.

Typical procedure for secondary amine catalyzed inverse electron-demand Diels-Alder reactions with aldehyde or ketones. A mixture of 3-methyl-6-phenyl-1,2,4,5-tetrazine (20 mg, 0.12 mmol), aldehyde or ketone (0.24 mmol) and pyrrolidine (0.6 mg, 0.12 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature for 30 minutes. After removal of the solvent *in vacuo*, the crude product was purified by chromatography on silica to afford the desired product.

3-Methyl-6-phenylpyridazine (4).[26] ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.06–8.04 (m, 2H), 7.75 (d, *J* 8.7 Hz, 1H), 7.53–7.46 (m, 3H), 7.38 (d, *J* 8.7 Hz, 1H), 2.73 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.6, 157.2, 136.5, 129.8, 128.9, 127.2, 126.9, 123.9, 22.1; MS-EI (*m/z*) 170; HRMS (EI): *m/z* calcd for $C_{11}H_{10}N_2$: 170.0846; found:170.0844.

1-Methyl-4-phenyl-6,7-dihydro-5H-cyclopenta(d)pyridazine (5). Oil. ^{1}H NMR ($CDCl_3$, 300 MHz): δ7.81 (d, *J* 7.3, 2H), 7.52-7.39 (m, 3H), 3.13 (t, *J* 7.2, 2H), 2.96 (t, *J* 7.2, 2H), 2.6 (s, 3H), 2.21-2.07 (m, 2H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 156.4, 155.7, 143.9, 141.6, 137.3, 128.9, 128.5, 128.4, 32.9, 31.0, 29.7, 23.9, 19.9. MS-EI (m+H) 211; HRMS(EI): calcd for $C_{14}H_{14}N_2$: 211.1235; found: 211.1231.

4,6-Dimethyl-3-phenylpyridazine (6). Off white solid. mp 80-82 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.57-7.53 (m, 2 H), 7.50-7.42 (m, 2H), 7.21 (s, 1H), 2.70 (s, 1H), 2.31 (s, 1H).^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.1, 158.3, 137.1, 135.7, 219.1, 128.6, 128.4, 128.3, 21.8, 19.6; MS-EI (*m/z*) 184; HRMS(EI): *m/z* calcd for $C_{12}H_{12}N_2$: 184.100; found: 184.0995.

3,6-Dimethyl-6-phenylpyridazine (7).[27] ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (dd, *J* 7.0, 0.6 Hz, 2H), 7.57 (s, 1H), 7.53-7.44 (m, 3H), 2.70 (s, 3H), 2.36 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 160.1, 158.2, 137.2, 135.7, 129.1, 128.7, 128.5, 128.3, 21.8, 19.6; MS-EI (*m/z*) 184; HRMS(EI): *m/z* calcd for $C_{12}H_{12}N_2$: 184.100; found: 184.0995.

4-Butyl-3-methyl-6-phenylpyridazine (3). Light yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.04 (dd, *J* 7.2, 0.7 Hz, 2H), 7.54 (s, 1H), 7.51-7.42 (m, 3H), 2.71 (s, 3H), 2.64 (t, *J* 7.0 Hz, 2H), 1.68-1.58 (m, 2H), 1.50-1.38 (m, 2H), 0.98 (t, 7.3 Hz, 3H).^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.1, 157.7, 140.9, 136.7, 129.5, 128.9, 126.9, 123.1, 31.8, 30.6, 22.5, 19.7, 13.9; MS-EI (*m/z*) 226; HRMS(EI): *m/z* calcd for $C_{12}H_{12}N_2$: 226.1470; found: 226.1466.

4-Benzyl-3-methyl-6-phenylpyridazine (8). Off white solid. mp 128-133 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.99 (d, *J* 6.3 Hz, 2H), 7.52-7.42 (m, 4H), 7.38-7.29 (m, 3H), 7.15 (d, *J* 7.2, 2H), 4.02 (s, 2H), 2.7 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 158.24, 157.71, 140.89, 136.73, 129.52, 128.85, 126.89, 123.11, 31.76, 30.62, 22.54, 19.74, 13.88; MS-EI (*m/z*) 260; HRMS(EI): *m/z* calcd for $C_{18}H_{16}N_2$: 260.1316; found: 260.1313.

NT-tetrazine (10). To compound **9** (104.6 mg, 0.34 mmol) in acetonitrile (10 mL) dichlorotetrazine (50.5 mg, 0.34 mmol) and TEA (100 µL) were added. The reaction was stirred at room temperature for 12 h. The product was poured in water (30 mL) and filtered to obtain a dark red solid, which was purified by chromatography on silica using CH_2Cl_2:CH_3OH (10:0.1-10:0.5) gradient to obtain **10** as dark red solid (106 mg, 75%). mp 221-223 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.44 (d, *J* 8.0 Hz, 1H), 8.33 (d, *J* 8.0 Hz, 1H), 8.02 (d, *J* 8.0 Hz, 1H), 7.51 (t, *J* 8.0 Hz, 1H), 7.07 (brs, 1H), 6.66 (d, *J* 8.0 Hz, 1H), 6.63 (brs, 1H), 4.13-4.04 (m, 4H), 3.77 (t, *J* 7.1 Hz, 2H), 1.70-1.60 (m, 2H), 1.46-1.35 (m, 2H), 0.94 (t, *J* 7.1 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 164.5, 164.1, 161.9, 161.1, 148.9, 134.1, 131.2, 129.5, 126.0, 125.0, 122.9, 120.2, 110.8, 104.1, 43.7, 40.6, 40.1, 30.9, 30.2, 20.4, 13.9; MS-EI (*m/z*) 426; HRMS(EI): *m/z* calcd for $C_{20}H_{20}ClN_7O_2$: 426.1445; found: 426.1451.

NT-diazine (11). NT-tetrazine **10** (50.0 mg, 0.18 mmol) in dry CH_2Cl_2 (50.0 mg, 0.18 mmol) was mixed with phenylpropionaldehyde (15.8 mg, 0.18 mmol) and diethylamine (18 µL, 0.18 mmol). The mixture was stirred at room temperature for 24 h and the solvent removed *in vacuo*. The crude green fluorescent mixture was purified on silica gel column with CH_2Cl_2:CH_3OH (20:1) to obtain **11** as yellow solid (25 mg, 38%). mp 103-106 °C ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.57 (d, *J* 8.3 Hz, 1H), 8.42 (d, *J* 8.3 Hz, 1H), 8.32 (d, *J* 8.3 Hz, 1H), 8.21 (brs, 1H), 7.65 (t, *J* 7.7 Hz, 1H), 7.14 (d, *J* 7.0 Hz, 2H), 7.11-7.01 (m, 3H), 6.48 (d, *J* 8.3 Hz, 1H), 4.15 (t, *J* 7.5 Hz, 2H), 3.87 (s, 2H), 3.50 (brs, 2H), 3.29 (s, 1H), 3.06-2.95 (m, 1H), 2.89-2.80 (m, 1H), 2.55 - 2.29 (m, 3H), 2.19-2.04 (m, 2H), 1.77-1.64 (m, 2H), 1.52-1.37 (m, 2H), 0.96 (t, *J* 7.1 Hz, 3H), 0.64 (t, *J* 8.0 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz). δ 164.8, 164.4, 157.9, 150.4, 135.9, 134.4, 134.1, 129.7, 129.1, 128.8, 128.0, 127.3, 124.9, 122.6, 120.4, 109.5, 102.7, 43.1, 40.8, 39.7, 35.7, 30.1, 20.5, 13.9, 13.4; MS-EI (*m/z*) 587; HRMS(EI+H): *m/z* calcd for $C_{20}H_{20}ClN_7O_2$: 587.2901; found: 587.2896.

Acknowledgements

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