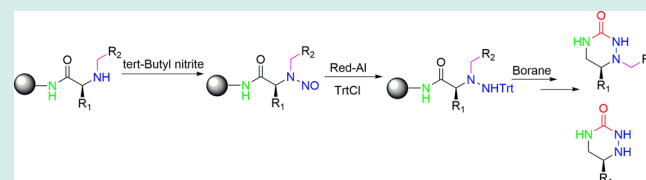


## Parallel Synthesis of 1,6-Disubstituted-1,2,4-triazin-3-ones on Solid-Phase

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## Supporting Information

**ABSTRACT:** A parallel solid-phase synthesis of 1,6-disubstituted-1,2,4-triazin-3-ones from MBHA resin is described. The reduction of resin-bound nitrosamino acids provides hydrazines efficiently without affecting the amide bond. The trityl protected hydrazine is then reduced with borane, and cyclized with 1,1-carbonyldiimidazole. The desired products are cleaved from their solid support and obtained in good yield and purity. This methodology is of value for the rapid parallel



preparation of these potentially bioactive molecules.

**KEYWORDS:** solid-phase, 1,2,4-triazinones, N-nitroso, hydrazine, reduction, cyclization

Solid-phase organic synthesis (SPOS) is a pivotal technique for the rapid access to structurally diverse compounds in the drug discovery process.<sup>1</sup> One major focus of this field is on the synthesis of small bioactive molecules and their derivatives on the solid phase. In particular, heterocyclic compounds have received special attention due to their high degree of structural diversity and multiple applications in the treatment of human diseases.<sup>2,3</sup>

1,2,4-Triazinones are analogues of the pyrimidine bases of nucleic acids and an important class of molecules with several biological properties. For example, 1,2,4-triazin-3(2H)-ones have been shown to possess potential as anticancer,<sup>4</sup> antiviral<sup>5</sup> and antibacterial drugs.<sup>6</sup> Moreover, 1-aryl-2H,4H-tetrahydro-1,2,4-triazin-3-ones were reported as orally selective 5-lipoxygenase (5-LO) inhibitors.<sup>7</sup> However, to the best of our knowledge, no examples of 1,6-disubstituted-1,2,4-triazin-3-one have been reported in the literature.

In the past decades, the N-nitroso group (N-NO) has attracted much attention because of its pharmaceutical properties and its synthetic utility.<sup>8</sup> It can be used synthetically to generate an N–N bond, as well as a hydrazine linker. There are several reagents that can reduce the N-NO group in order to obtain hydrazine, including Zn/AcOH,<sup>9</sup> LiAlH<sub>4</sub>,<sup>10</sup> or titanium trichloride (TiCl<sub>3</sub>)<sup>11</sup> in solution phase, and LiAlH<sub>4</sub><sup>8b,12</sup> or DIBAL<sup>13</sup> on solid-phase. We have found that LiAlH<sub>4</sub> or DIBAL do not selectively afford hydrazine efficiently when an amide bond is present, even at low temperatures, such as 0 °C with DIBAL (data not shown), since the amide bond will also be reduced. Red-Al is an alternative reductive reagent to LiAlH<sub>4</sub>, and it is much safer and more convenient for storage. While Lee et al.<sup>14</sup> have reported the reduction of amide bonds with Red-Al as part of their effort to obtain [1,2]-diamines at room temperature, it was believed that the conditions could be optimized to selectively reduce N-NO over amide bonds.

Herein, a synthetic strategy in which Red-Al is highly efficient and selective at reducing N-NO over amides has been developed (Scheme 1). From the LC-MS data, the crude product **A** was obtained with 95% purity ( $t_R = 5.214$  min). Only 3% of the amide bond was reduced ( $t_R = 6.227$  min) (Figure 1). The isolated yield of **A** was 88%.

Moreover, through the use of a reductive alkylation, diverse substitutions were generated on the N<sup>1</sup> position of hydrazine (Table 1). As a note, it was observed that the 4-OBn-Bn group was cleaved from the hydrazine moiety by HF (Scheme 2), which provided us a new method for obtaining N<sup>1</sup> (H)-hydrazine. The isolated yield of **B** was 82%. Additionally, **B** was characterized by LC-MS, HRMS, <sup>1</sup>H NMR, and <sup>13</sup>C NMR (see Supporting Information).

To extend the practical value of this methodology for the production of a hydrazine moiety, it was applied to the synthesis of 1,6-disubstituted-1,2,4-triazin-3-ones (Scheme 3). Starting from *p*-methylbenzhydramine (MBHA) resin **1**, a Boc-amino acid was coupled to the resin using a standard DIC/HOBt procedure to generate the resin bound amino acid **2**. After removal of the Boc group with 55% TFA in DCM, a reductive alkylation was performed in order to introduce a secondary diversity<sup>15</sup> (**3**). Subsequent reaction with *tert*-butyl nitrite led to the resin-bound nitroso compound **4**. The hydrazine **5** was then generated by reducing **4** with Red-Al at room temperature. The hydrazine was protected with a Trt group because of its reported instability to BH<sub>3</sub>/THF.<sup>13</sup> The corresponding resin-bound product **7** was treated with 2% TFA/DCM and cyclized with 1,1-carbonyldiimidazole, which was then released from the resin by HF at 0 °C. Afterward, the

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## Scheme 1. Synthesis Approach for Hydrazine on Solid-Phase

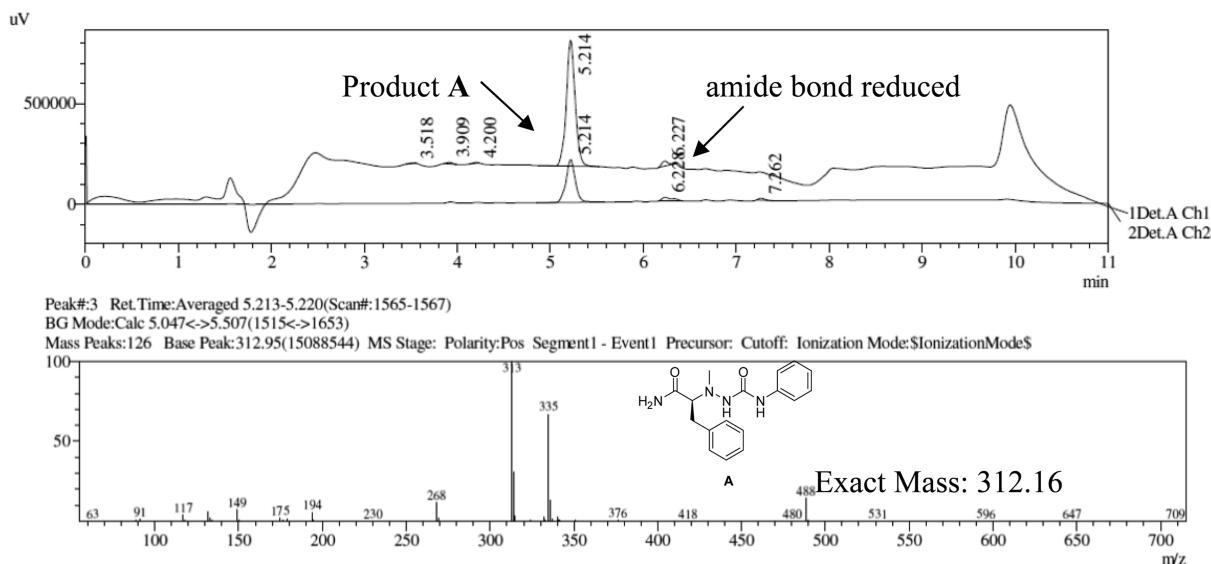
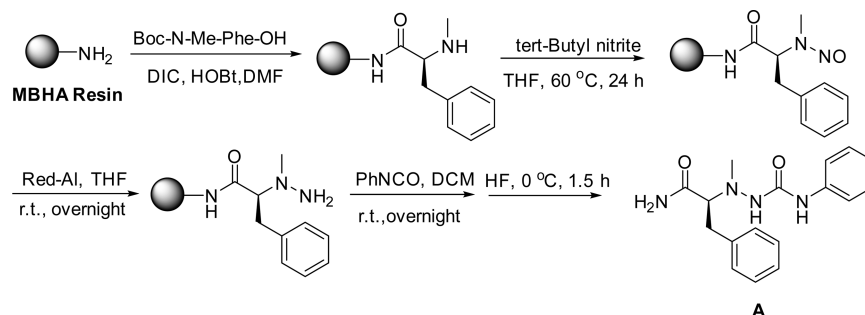


Figure 1. LC-MS data of crude product A.

final products **9** (or **9'**) were obtained in good yields and purity.

As outlined in Table 1, several amino acids and different aldehydes were accessed for viability under the synthetic conditions. When  $R_2$  was Phenyl, amino acids with an aromatic side chain generated products with slightly higher purity than those with alkyl side chains according to the HPLC data of the crude products (**9a–9e** to **9f–9i**, except **9d**, which may be due to the 1-naphthyl hindering the cyclization). When  $R_2$  contained the electron-withdrawing group 2-fluoro on the phenyl ring, the purity was excellent (**9l** and **9m**). However, the situation was different when 4-bromo was present (**9j** and **9k**). This may be the result of dehalogenation of the bromo during the Red-Al reduction reaction. When the electron-donating group 4-OBn was present on the phenyl ring (**9'a–9'e**), the entire 4-OBn-Bn group was cleaved from the product during the HF step. Low purity products were obtained for these compounds (**9'a–9'e**). The overall yield for the analogs tested under the described conditions ranged from 56–82% (Table 1).

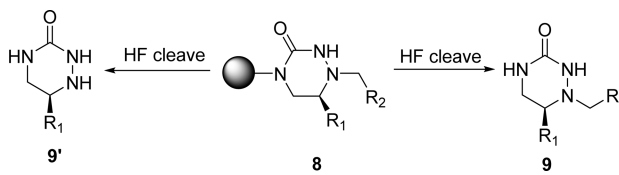
In conclusion, the reduction of an N-NO group on the solid-phase affords a facile approach for producing amino acids with hydrazine termini and a reductive alkylation provides a second diversity position. Moreover, these synthetic methods can be applied to the efficient parallel synthesis of novel 1,6-disubstituted-1,2,4-triazin-3-ones. This methodology is of value for the rapid parallel preparation of these potentially bioactive molecules in drug discovery.

## EXPERIMENTAL PROCEDURES

**General Methods.** Reagents were obtained from commercial sources and used without any further purification. Solvents were purchased from Sigma-Aldrich and were used directly. The isolated yields were based on the manufacturer's loading of MBHA resin. LC-MS (APCI and ESI) were recorded on a Shimadzu LCMS-2010EV at LCQ mass spectrometer (ThermoQuest Corporation) using a Phenomenex Luna 5  $\mu$  C18, 100 Å, 150  $\times$  4.60 mm 5  $\mu$ m column running a 5–95 (H<sub>2</sub>O/CH<sub>3</sub>CN; 0.1% formic acid) gradient over 6 min. Preparative RP-HPLC was performed on a Shimadzu LC-8A preparative HPLC using a Phenomenex Luna 5  $\mu$  C18 (2) 100 Å column (75  $\times$  21.2 mm, 5  $\mu$ m). High resolution mass spectra were measured on an Agilent 1290 HPLC-6224 Time of Flight Mass Spectrometer. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra were recorded at 400 MHz on a Bruker Advance spectrometer with a 5-mm inverse gradient probe.



**General Procedures for the Synthesis 1, 6-Disubstituted-1, 2, 4-Triazin-3-Ones (9a–9o, 9'a–9'e).** One hundred milligrams of MBHA resin (1.1 mequiv/g) was contained in a polypropylene mesh packet. Following neutralization with 5% diisopropylethylamine (DIEA), the resin was coupled with Boc-amino acid, hydroxybenzotriazole (HOBT, 6.0 equiv, 0.1 M) and diisopropylcarbodiimide (DIC, 6.0 equiv, 0.1 M) in DMF at room temperature for 2 h. Upon removal of the Boc group with 55% TFA in DCM (30 min), the resin was washed with DCM (2 times), neutralized with a solution of 5% DIEA in DCM. Reductive alkylation was performed according to the general methods. AcOH (0.11 mL) and trimethyl orthoformate (TMOF) (0.55 mL) were added to a mixture of resin in DCM (8.1 mL) and MeOH (2.2 mL). The appropriate aromatic aldehyde (10 equiv) was added to the above solution and shaken for 20 min. NaCNBH<sub>3</sub> (69 mg, 10 equiv) in DMF (1.1 mL) was added and the mixture was

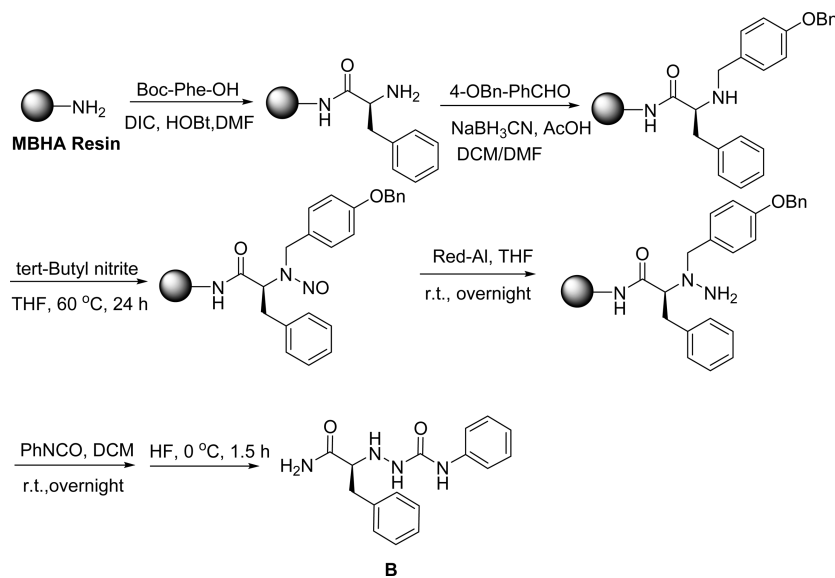
Table 1. Individual Products of 1,6-Disubstituted-1,2,4-triazin-3-ones



compound	R <sub>1</sub>	R <sub>2</sub>	mass <sup>a</sup> [M + H] <sup>+</sup>	t <sub>R</sub> <sup>b</sup> (min)	purity <sup>c</sup> (%)	HRMS [M + H] <sup>+</sup>	yield <sup>d</sup> (%)
9a	Benzyl	Phenyl	281.9	5.60	92	282.1606	64
9b	4-F-Benzyl	Phenyl	299.9	5.72	90	300.1514	71
9c	4-OCH <sub>3</sub> Benzyl	Phenyl	311.9	5.58	88	312.1645	69
9d	1-Naphthyl-CH <sub>2</sub> <sup>e</sup>	Phenyl	332.0	6.22	70	332.1761	56
9e	2-Naphthyl-CH <sub>2</sub> <sup>f</sup>	Phenyl	331.9	6.26	92	332.1759	64
9f	H	Phenyl	191.9	4.01	88	192.1133	64
9g	CH(CH <sub>3</sub> ) <sub>2</sub>	Phenyl	234.0	5.43	80	234.1604	60
9h	CH <sub>2</sub> CH(CH <sub>3</sub> ) <sub>2</sub>	Phenyl	248.0	5.73	85	248.1758	68
9i	CH <sub>3</sub>	Phenyl	205.9	4.33	81	206.1294	70
9j	Benzyl	4-Br-Phenyl	359.7	6.18	69	360.0712	60
			361.7			362.0693	
9k	CH <sub>3</sub>	4-Br-Phenyl	283.7	5.03	55	284.0399	65
			285.7			286.0381	
9l	Benzyl	2-F-Phenyl	299.9	5.63	88	300.1515	70
9m	CH <sub>3</sub>	2-F-Phenyl	223.9	4.37	93	224.1197	82
9n	Benzyl	2-Naphthyl	332.0	6.28	80	332.1761	65
9o	CH <sub>3</sub>	2-Naphthyl	255.9	5.21	90	256.1447	68
9'a	Benzyl	4-OBnPhenyl	191.9	3.98	40	192.1136	68
9'b	4-F-Benzyl	4-OBnPhenyl	209.9	4.16	55	210.1042	70
9'c	4-OCH <sub>3</sub> Benzyl	4-OBnPhenyl	221.9	4.01	28	222.1139	64
9'd	1-Naphthyl-CH <sub>2</sub> <sup>e</sup>	4-OBnPhenyl	241.9	4.72	58	242.1293	61
9'e	2-Naphthyl-CH <sub>2</sub> <sup>f</sup>	4-OBnPhenyl	241.9	4.80	63	242.1298	61

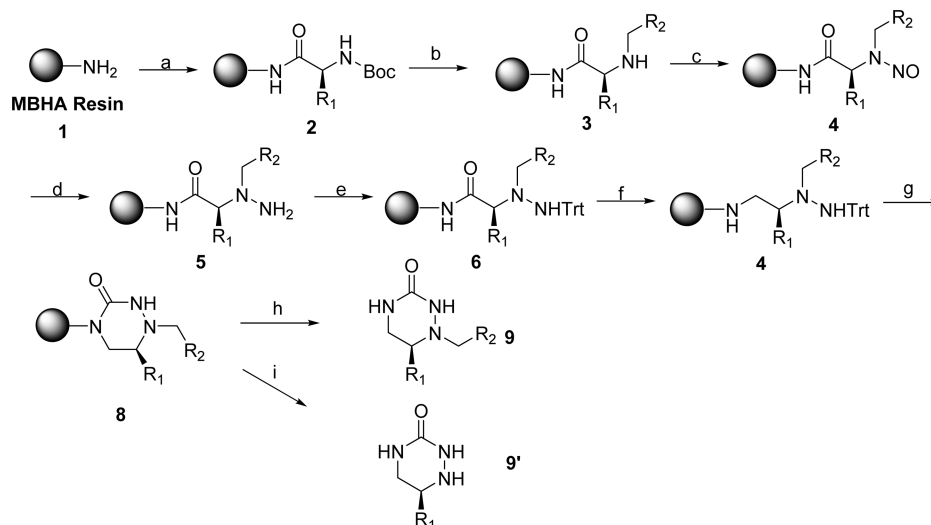
<sup>a</sup>Confirmed by mass spectra (ESI). <sup>b</sup>Retention times from analytical RP-HPLC profile (UV detection at 214 and 254 nm). <sup>c</sup>The purity of the crude material was estimated by the peak area from analytical RP-HPLC traces at  $\lambda = 254$  and 214 nm. <sup>d</sup>Isolated yield based on manufacturer's loading of

MBHA resin. <sup>e</sup>1-Naphthyl-CH<sub>2</sub>:  <sup>f</sup>2-Naphthyl-CH<sub>2</sub>: 

Scheme 2. Synthesis Approach for N<sup>1</sup> (H)-Hydrazine on Solid-Phase

shaken for 1 h at room temperature. The resin was then washed with DMF, DCM, 5% DIEA/DCM, DCM and MeOH, and dried in the hood. The resulting resin bound amine was treated with 0.5 M tert-Butyl nitrite in THF at 60 °C for 24 h. The bag was washed with THF, DCM, MeOH, and dried in vacuo. The resulting resin-bound

nitrosamino acid was then reduced with 0.5 M Red-Al (70% in toluene) in THF. The reduction solution was poured off and quenched with methanol before adding to waste, and then washed bags with THF and MeOH several times. The corresponding resin-bound hydrazine was reacted with TrtCl (10 equiv) and DIEA (20

Scheme 3. Synthesis of 1,6-Disubstituted-1,2,4-triazin-3-ones<sup>a</sup>

<sup>a</sup>Reagents and Conditions: (a) (i) prewash with 5% DIEA/DCM; (ii) Boc-AA-OH, DIC, HOBT, DMF, r.t., 2 h; (b) (i) 55%TFA/DCM; (ii) reductive alkylation, R<sub>2</sub>CHO; (c) 0.5 M *tert*-butyl nitrite/THF, 60 °C, 24 h; (d) Red-Al, THF, r.t., overnight; (e) TrtCl, DIPEA, DMF, DCM; (f) BH<sub>3</sub>/THF, 60 °C; (g) (i) 2% TFA/DCM; (ii) 1,1-carbonyldiimidazole, DCM; (h) HF, 0 °C, 1.5 h; (i) HF, 0 °C, 1.5 h.

equiv) in 0.1 M 10% DMF/DCM. The resin was washed with DMF and DCM twice. After the Trt protection, the resin was reduced in BH<sub>3</sub>/THF at 60 °C for four days and then with piperidine at 60 °C for one day. The bags were then washed with DMF (twice), DCM (four times) and MeOH (twice). After removal of the Trt group in 2% TFA/DCM (3 × 10 min), the resin was cyclized with 1,1-carbonyldiimidazole (6 equiv) in DCM. The final product was then released from the resin by anhydrous HF in the presence of 0.5% anisole at 0 °C for 1.5 h. The product was extracted by 95% acetic acid in water. After lyophilization, the product of 1,6-disubstituted-1,2,4-triazin-3-one was obtained. The crude product was purified by preparative HPLC and characterized by LC-MS under ESI conditions, HRMS and NMR spectroscopy.

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental data for compounds A, B, 9a–9o, 9'a–9'b, and <sup>1</sup>H, <sup>13</sup>C NMR spectra. This material is available free of charge via Internet at <http://pubs.acs.org>.

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### Notes

The authors declare no competing financial interest.

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