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RESEARCH ARTICLE

ROUTE DEVELOPMENT, ANTIVIRAL STUDIES, FIELD EVALUATION AND TOXICITY OF AN ANTIVIRAL PLANT PROTECTANT NK0238

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KEYWORDS

antiviral plant protectant, antiviral in the greenhouse, field evaluation, L-trp-NCA, synthesis optimization, toxicity tests

HIGHLIGHTS

- Developed a two-step synthetic route to anti-plant-virus candidate NK0238.
- NK0238 exhibited a broad antivirus spectrum in greenhouse.
- NK0238 showed comparable antivirus activities as controls in field trials.
- NK0238 was safe to birds, fish, bees and silkworms.
- NK0238 has a very good prospect in commercial development.

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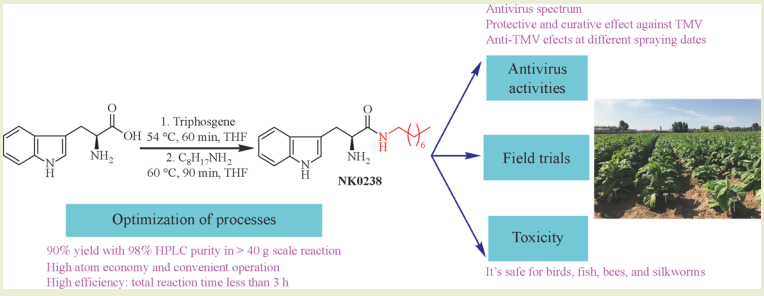
GRAPHICAL ABSTRACT



ABSTRACT

It has previously been shown that tryptophan, the biosynthesis precursor of *Peganum harmala* alkaloids, and its derivatives have anti-TMV activity both *in vitro* and *in vivo*. Further exploration of this led to the identification of NK0238 as a highly effective agent for the prevention and control of diseases caused by plant viruses, but the existing routes are unsuitable for its large-scale synthesis. This study optimized a route for two-step synthesis of this virucide candidate via reaction of L-tryptophan with triphosgene to produce L-tryptophan-*N*-carboxylic anhydride, which then reacts with *n*-octylamine to give NK0238 at up to 94% yield and nearly 97% HPLC purity. In addition, the route was used for the preparation of NK0238 on a > 40 g scale permitting further assessment of its antivirus activity in the greenhouse and field experiments, and toxicity tests. NK0238 exhibited useful antiviral activities against a variety of viruses both in greenhouse and field experiments. The toxicity tests showed that NK0238 was not acutely toxic to birds, fish, honey bees and silkworms. The optimized route provides a solid foundation for its large-scale synthesis and subsequent efficacy and toxicity studies, its excellent activity and safety make NK0238 a promising drug candidate for further development.

1 INTRODUCTION

Plant diseases caused by viruses seriously damage food crops and cash crops around the world and are extremely difficult to prevent and control[1]. Considerable research on compounds with activity against plant-virus diseases has been conducted [2–6]. Song et al. created an efficient antiviral dufulin[7,8]. Hao et al. found lycorine and eudesmanolides exhibited anti-phytovirus activity[9,10]. Wang et al. found series of marine natural products could be used as anti-plant-virus lead compounds[11–14]. Xiaet al.[15] found genipin and their derivatives and Li et al.[16] found cerbinal and their derivatives both exhibited anti-virus activity. Although considerable progress in developing resistance to plant viruses has been made, few satisfactory plant-virus inhibitors and even fewer therapeutic agents have been developed.

It is increasingly important and growing research focus to find drug candidates from natural products[17,18]. Natural products posses many of the properties that can make them useful drug candidates, including structural diversity, specificity and novel modes of action[19]. However, natural products also have some disadvantages, such as limited compound availability, high structural complexity and poor drug-likeness[20]. It is therefore important to explore newly identified and synthesized compounds or molecules with different targets from natural products and to develop new pesticides through rational biological design[21].

In our previous work, we reported for the first time that tryptophan, the biosynthesis precursor of *Peganum harmala* alkaloids, and its derivatives containing amide or ester moiety have useful anti-TMV (tobacco mosaic virus) activity both *in vitro* and *in vivo*[22]. Further exploration led to the identification of NK0238 (2-amino-3-(1H-indol-3-yl)-*N*-octyl-propionamide) as a highly effective agent for the prevention and control of diseases caused by plant viruses (Fig. 1). To undertake greenhouse or field evaluations and toxicity tests, large amounts of candidates compound are needed. Two routes to NK0238 have been reported, both of which use *N*-(3-(dimethylamino) propyl)propionimidamide hydrochloride as a condensing agent for the reaction of *n*-octylamine and N-protected tryptophan; the protecting group, which is necessary to prevent self-condensation of tryptophan, must be removed after the condensation step[22] (Fig. 2). The atom economy and yields of these three-step routes are low, and the raw materials are expensive, which makes the routes unsuitable for large-scale synthesis. Development of a route with operational simplicity, suitability for scale-up is urgently needed.

We speculated that *N*-carboxyanhydrides (NCAs), specifically α-amino acid-NCA, would be useful for this purpose. NCAs are reactive compounds that are widely used as monomers for the synthesis of peptides[23,24] and as platform chemicals for biomedical applications[25–27]. In addition, NCAs can be used to prepare reactive esters[28]. There are several known methods for preparing NCAs. The first synthesis was reported in 1906 by Leuchs, who synthesized α-amino NCAs from *N*-alkoxycarbonyl amino acid chlorides[29]. Subsequently, Kricheldorf developed a method involving reactions of *N*-alkoxy amino acids with $SOCl_2$, PBr_3 and other halogenating agents[30]. In addition, phosgene (carbonyl chloride)[31], diphosgene (trichloromethyl chloride)[32], triphosgene (bis(trichloromethyl) carbonate)[33], and di-*tert*-butyltricarbonate[34] act as cyclocarbonylation reagents

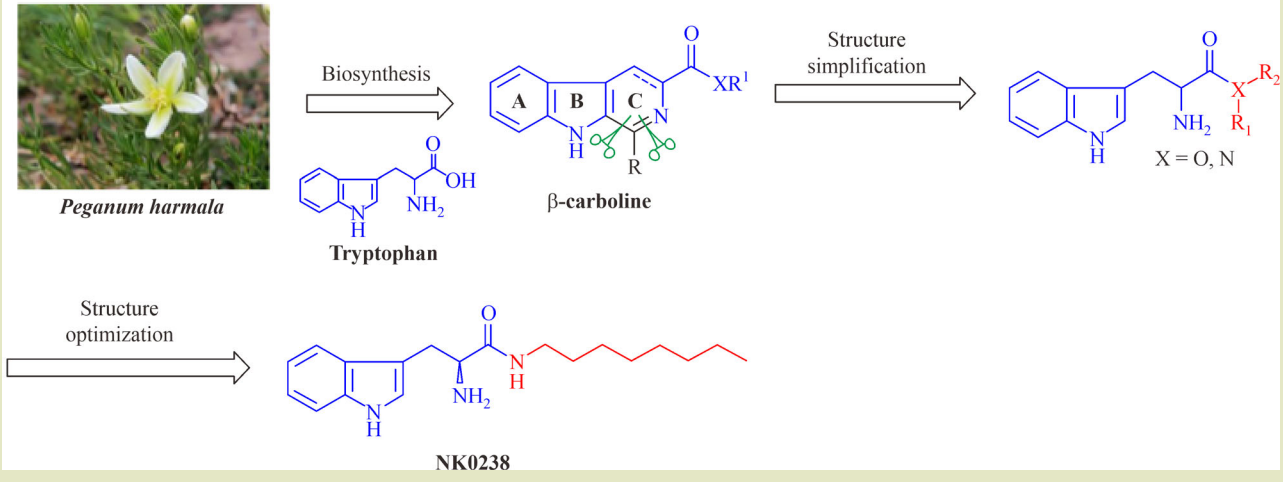


Fig. 1 Route for synthesis of the antiviral plant protectant, NK0238.

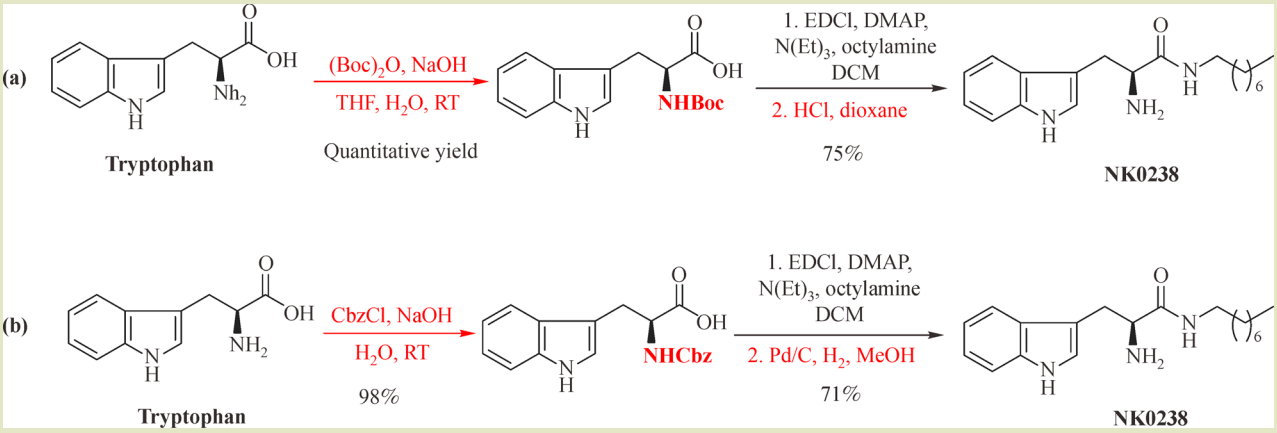


Fig. 2 Previously reported routes for the synthesis of the anti-plant-virus candidate, NK0238.

for the synthesis of NCAs from unprotected amino acids. We hypothesized that if we selected an appropriate cyclocarbonylation agent, reaction with unprotected L-tryptophan would generate L-trp-NCA, which could then react with *n*-octylamine to give NK0238 (Fig. 3). The only byproducts of this new route would be HCl and CO_2, little solid waste would be generated, and the route would be operationally simpler and less costly than the previously reported routes. In this work, we developed a two-step synthesis of this virucide candidate via reaction of L-tryptophan with triphosgene to produce L-tryptophan-*N*-carboxylic anhydride, which then reacts with *n*-octylamine to give NK0238. Then, we conducted antivirus assessemtns in greenhouse and field experiments, and toxicity tests.

2 MATERIALS AND METHODS

2.1 General

All reagents were purchased from commercial sources and were used as received. All anhydrous solvents were dried and purified according to standard techniques immediately before use. ^{1}H NMR and ^{13}C NMR spectra were measured on a 400 MHz spectrometer (Bruker, Billerica, MA, USA). Mass spectra were obtained with a Fourier transform ion cyclotron resonance mass spectrometer (IonSpec, 7.0T, IonSpec Co. Ltd., Lake Forest, CA, USA). Melting points were obtained on an X-4 binocular microscope melting point apparatus. HPLC was performed on an Agilent 1260 chromatograph equipped with a VP-ODS column (4.6 mm × 250 mm, 5 μm, Shimadzu Kyoto, Japan) under the following conditions: mobile phase, 80:20 (v/v) CH_3OH/H_2O containing 0.5% $NH_3{\cdot}H_2O$; flow rate, 1.0 mL·min^{-1}; column temperature, 40 °C; UV detection, 245 nm; detection time, 15 min.

2.2 Synthesis

The synthesis process optimization and synthetic routes are given in Tables 1–3 and Figs. 4–7.

2.2.1 Preparation of (S)-4-((1H-indol-3-yl)methyl)oxazolidine-2,5-dione (L-trp-NCA)

L-Tryptophan (30.6 g, 150 mmol) was weighed into a 1000 mL three-necked round-bottom flask, and anhydrous tetrahydrofuran (THF; 250 mL) was added. One neck of the flask was equipped with a reflux condenser fitted with a drying tube connected to a plastic tube, one end of which was immersed in weighed dilute aqueous NaOH; the other two necks of the flask

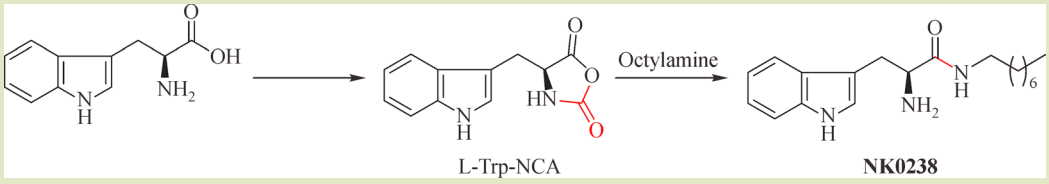


Fig. 3 Proposed new route for the synthesis of the anti-plant-virus candidate, NK0238.

Table 1 Synthesis of L-trp-NCA with triphosgene as the cyclocarbonylation agent in various solvents[a]

Entry	Solvent[b]	Appearance	Temperature (°C)	Time (min)
1	THF	clear	64	60
2	toluene	turbid	64	180
3	DCE	turbid	64	180
4	DCM	turbid	reflux	180

Note: [a]Amount of L-tryptophan was 15 mmol (3.06 g). [b]THF, tetrahydrofuran; DCE, 1,2-dichloroethane; and DCM, dichloromethane.

Table 2 Optimization of conditions for the first step[a]

Entry	Triphosgene (equiv.)	Temperature[b] (°C)	Time[c] (min)	Volume of DMF[d] (mL)	Yield[e] (%)
1	0.47	64	60	400	88
2	0.47	64	60	1000	88
3	0.47	64	60	100	76
4	0.47	64	60	0	67
5	0.43	64	60	400	87
6	0.50	64	60	400	88
7	0.47	64	45	400	87
8	0.47	64	75	400	81
9	0.47	54	70	400	90
10	0.47	46	190	400	82
11	0.47	40	270	400	83
12[f]	0.47	54	60	400	92
13[g]	0.47	54	65	400	92

Note: [a]Amount of L-tryptophan was 15 mmol (3.06 g). [b]Reaction temperature for preparation of L-trp-NCA. [c]Time required for the reaction mixture to clear. [d]DMF, *N,N*-dimethylformamide. [e]Isolated by column chromatography on silica gel with dichloromethane/methanol (50/1) as eluent. [f]Volume of THF was 40 mL. [g]Volume of THF was 50 mL.

Table 3 Optimization of reaction conditions for the second step[a]

Entry	Temperature (°C)	Amount of *n*-octylamine (equiv.)	Yield[b] (%)	Purity[c] (%)
1	25	1.50	92	89.1
2	25	1.75	92	91.4
3	25	2.00	93	95.1
4	0	2.00	91	91.1
5	40	2.00	94	96.3
6	50	2.00	94	96.9
7	60	2.00	94	97.2

Note: [a]The amount of L-tryptophan was 15 mmol (3.06 g). [b]Isolated by column chromatography on silica gel with dichloromethane/methanol (50/1) as eluent. [c]The purity of NK0238 was determined by an external standard method.

were equipped with thermometer and an addition funnel. The THF solution was heated to 54 °C, and a solution of triphosgene (20.9 g, 70.5 mmol) in 150 mL of anhydrous THF was added dropwise over the course of 20 min. The reaction mixture was then stirred at 54 °C for 40 min, during which time it changed from turbid to clear; HCl was released during this stage of the reaction. After the mixture was cooled to room temperature, DMF (4 mL) was added under a nitrogen flow, and the resulting

solution was stirred until there was no change in the mass of the dilute aqueous NaOH solution (15 min; about 13.1 g of HCl was recovered). Then the reaction mixture was transferred to a single-necked round-bottom flask (1 L) and concentrated in vacuo in a water bath kept at a temperature below 30 °C to give crude L-trp-NCA. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.00 (s, 1H), 9.07 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.14 (s, 1H), 7.07 (t, J = 7.4 Hz, 1H), 6.99 (t, J = 7.3 Hz, 1H), 4.78 (t, J = 4.9 Hz, 1H), 3.20 (dd, J = 15.1, 5.1 Hz, 1H), 3.12 (dd, J = 15.1, 4.7 Hz, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.7, 152.3, 136.4, 127.6, 124.9, 121.5, 119.0, 111.8, 107.5, 99.9, 58.7, 26.9.

2.2.2 Preparation of NK0238

A round-bottom flask (2 L) was charged with *n*-octylamine (49.6 mL, 300 mmol) and anhydrous THF (400 mL), and the resulting solution was heated to 50 °C with stirring. Then a solution of crude L-trp-NCA in anhydrous THF (600 mL) was added dropwise over the course of 1.5 h; CO_2 is released during this process, so the rate of addition must be carefully controlled. After the addition of L-trp-NCA was complete, the progress of the reaction was monitored by TLC. When TLC indicated that the reaction was complete, the solvent was removed in vacuo, the residue was dissolved in ethyl acetate (150 mL), and 1.1 equiv. (165 mmol) of HCl in ethyl acetate solution (1.43 mol·L^{-1}, 115 mL) was added over the course of 5 min. Then, the *n*-octylamine hydrochloride produced was removed by filtration (24.0 g, recovery rate 97%). The pH of the filtrate was then adjusted to ~ 4 with HCl in ethyl acetate (1.43 mol·L^{-1}, 125 mL) and filtrate was allowed to stand for 8 h at room temperature. The large amount of NK0238 hydrochloride that precipitated was collected by filtration. The filtrate was concentrated in vacuo to 50 mL, and *n*-hexane (30 mL) was slowly added dropwise. After the resulting solution stood for 6 h, the precipitated NK0238 hydrochloride filtered off. Two portions of NK0238 hydrochloride were combined (48.6 g, 138 mmol) and neutralized with aqueous NaOH (0.75 mol·L^{-1}, 200 mL). The aqueous phase was extracted with ethyl acetate (100 × 3 mL), and the combined organic phases were washed with saturated brine (200 mL), dried with anhydrous sodium sulfate (6 g), and filtered. The filtrate was evaporated to give NK0238 (43.0 g, 91%) as a light-yellow solid with 98% HPLC purity (mp 46–47 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.34 (s, 1H), 7.67 (d, J = 7.9 Hz, 1H), 7.37 (d, J = 8.1 Hz, 1H), 7.23–7.18 (m, 1H), 7.14–7.10 (m, 1H), 7.07 (d, J = 2.2 Hz, 1H), 3.71 (dd, J = 9.0, 4.2 Hz, 1H), 3.38 (d, J = 4.0 Hz, 1H), 3.24 (q, J = 6.5 Hz, 2H), 2.91 (dd, J = 14.5, 9.0 Hz, 1H), 1.45 (s, 4H), 1.26 (s, 8H), 0.88 (t, J = 6.9 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 136.5, 127.5, 123.2, 122.1, 119.4, 118.9, 111.6, 111.3, 55.6, 39.1, 31.8, 30.9, 29.5, 29.2, 29.2, 26.9, 22.6, 14.1. HRMS(ESI) calculated for $C_{19}H_{29}N_3O$ [M + H]$^+$, 315.2311; found 315.2317.

2.2.3 Data for bissulfonylated byproduct

The byproduct was obtained as a white solid by means of column chromatography on silica gel with 50:1 dichloromethane/methanol as the eluent (mp 166–167 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.86 (s, 1H), 10.80 (s, 1H), 8.01 (d, J = 7.9 Hz, 1H), 7.82 (s, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.32–7.29 (m, 2H), 7.14 (s, 1H), 7.08–6.95 (m, 4H), 6.90 (t, J = 7.2 Hz, 1H), 4.51 (d, J = 6.6 Hz, 1H), 3.44 (s, 1H), 3.07–2.87 (m, 5H), 2.64 (dd, J = 13.9, 8.6 Hz, 1H), 1.28–1.16 (brs, 12H), 0.84 (d, J = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 174.6, 171.4, 136.7, 136.5, 127.97, 127.95, 124.3, 124.0, 121.4, 121.2, 119.0, 119.0, 118.7, 118.6, 111.8, 111.7, 111.0, 110.1, 55.6, 53.3, 39.1, 31.8, 31.0, 29.4, 29.2, 29.1, 28.7, 26.8, 22.6, 14.4. HRMS(ESI) calculated for $C_{30}H_{40}N_5O_2$ [M + H]$^+$, 502.3177; found 502.3180.

2.3 Bioassay

Ningnanmycin (Alta Scientific Co., Ltd., Tianjin, China), amino-oligosaccharins (Topscience Co., Ltd., Rizhao, Shandong, China), moroxydine hydrochloride-copper acetate (Shanxi Yonghe Lifeng Chemical Co., Ltd., Taiyuan, Shanxi, China). Each test was repeated three times at 25±1 °C. Active effect expressed in percentage scale of 0–100 (no activity to total inhibition). The results are presented as mean±standard errors. Detailed procedures for the antivirus and field experiments were given in the Supplementary Information.

3 RESULTS AND DISCUSSION

3.1 Synthesis process optimization

3.1.1 Optimization of conditions for preparation and post-reaction treatment of L-trp-NCA

We optimized the conditions for the synthesis of NK0238 by focusing on two main points: (1) the preparation and post-reaction treatment of L-trp-NCA and (2) the reaction of L-trp-NCA with *n*-octylamine (Fig. 3).

First, we attempted to synthesize L-trp-NCA by means of a reaction between L-tryptophan and phosgene, which was produced by phenanthridine-catalyzed reaction of triphosgene[35] and bubbled into a solution of L-tryptophan in a separate flask (details of the reaction mechanism is shown in supplementary materials, Fig. S1). However, these conditions

produced a turbid reaction mixture, regardless of the solvent (THF or dichloromethane), the reaction temperature (room temperature or reflux temperature), or the amount of triphosgene (1.2–1.8 equiv.) (details of the screening conditions are given in supplementary materials, Table S1). The turbidity suggested that these conditions rapidly produced a large amount of phosgene, which in turn resulted in the accumulation of a large amount of HCl in the reaction medium. Therefore, the HCl salt of L-tryptophan formed, which inhibited the subsequent reaction with *n*-octylamine[26].

To control the rate of phosgene production, we tried using triphosgene to prepare L-trp-NCA (details of the reaction mechanism is shown in supplementary materials, Fig. S1) under a variety of conditions (Fig. 4 and Table 1). Screening of several solvents revealed THF to be the best choice. In an attempt to facilitate removal of the generated HCl from the reaction mixture, we heated it to 64 °C, which is close to the boiling point of THF. When a THF solution of tryptophan was treated with 0.47 equiv. of triphosgene for 60 min at 64 °C, the reaction mixture cleared, indicating that almost all of the tryptophan had been converted to L-trp-NCA (entry 1). Although L-tryptophan was poorly soluble in THF, L-trp-NCA dissolved in THF; therefore, observing whether the reaction mixture changed from turbid to clear was a feasible strategy for determining whether the reaction was complete[32].

Having determined the optimal solvent and temperature, we explored additional conditions for the reaction of L-tryptophan and triphosgene with the goal of optimizing the isolated yield of NK0238 (Fig. 5 and Table 2). Given that L-trp-NCA is difficult to separate and purify[32], we used the crude L-trp-NCA in the subsequent reaction with *n*-octylamine. To prevent any residual triphosgene from interfering with this subsequent reaction, we added *N*,*N*-dimethylformamide (DMF) to decompose the excess triphosgene after complete conversion of L-tryptophan to L-trp-NCA. Removal of the solvent gave crude L-trp-NCA, which was allowed to react with 1.5 equiv. of *n*-octylamine in THF at room temperature for 90 min. This procedure gave an 88% isolated yield of NK0238, regardless of whether the amount of DMF was 400 μL or 1 mL (entries 1 and 2). Reducing the amount of DMF to 100 uL or eliminating it altogether (entries 3 and 4, respectively) was detrimental to the yield. Using another amine quencher, such as pyridine or triethylamine, provided no benefit (details of the screening conditions are given in supplementary materials, Table S2). Therefore, in all subsequent reactions, 400 μL of DMF was used to quench the residual triphosgene. Next, we studied the effect of the amount of triphosgene on the yield of NK0238 (Table 2, entries 1, 5 and 6). Decreasing the amount to 0.43 equiv. or increasing it to 0.50 equiv. had little effect on the yield; therefore, we used 0.47 equiv. of triphosgene in all subsequent reactions.

We also studied the effect of reaction time on the yield of NK0238 (Table 2, entries 1, 7 and 8). If DMF was added after 45 min (when the reaction mixture cleared) instead of after 60 min, there was little difference in yield (88% versus 87%); however, if DMF was added after 75 min, the yield decreased to 81%. This decrease was probably due to the instability of L-trp-NCA, which decomposed with increasing reaction time. This finding suggests that adding the DMF as soon as the reaction mixture cleared was optimal.

Given that temperature influences the rate of the reaction

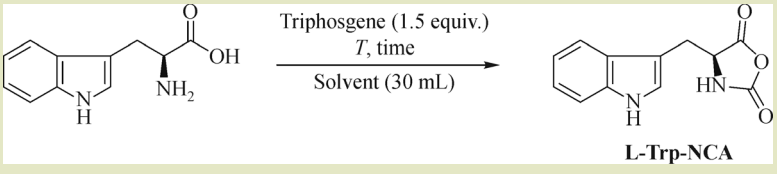


Fig. 4 Solvent screening for the synthesis of L-Trp-NCA with triphosgene as the cyclocarbonylation agent

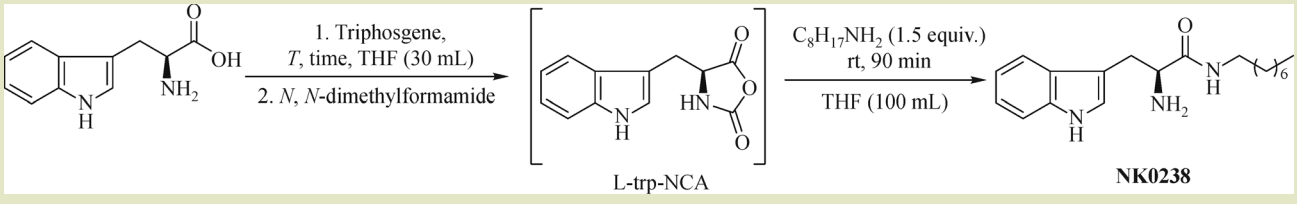


Fig. 5 Optimization of conditions for the first step.

between L-tryptophan and triphosgene, the rate of HCl generation, and thus the amount of L-tryptophan hydrochloride, we tested various reaction temperatures (Table 2, entries 1 and 9–11). Of the tested temperatures, 54 °C gave the highest yield; at this temperature, the reaction mixture became clear at 70 min, and the product yield was 90% (entry 9). Finally, we explored the effect of the L-tryptophan concentration (Table 2, entries 1, 12 and 13). When the concentration was decreased from 0.5 to 0.375 mol·L^{-1} (entry 12), the yield of NK0238 increased to 92%, but decreasing the concentration further (to 0.30 mol·L^{-1}) had no effect on the yield (entry 13).

3.1.2 Optimization of conditions for reaction of L-trp-NCA with *n*-octylamine

Using the optimized conditions for the first step (Table 2, entry 12), we then determined the optimal amount of *n*-octylamine for the second step (Fig. 6 and Table 3, entries 1–3). When 2.00 equiv. of *n*-octylamine was allowed to react with L-trp-NCA at 25 °C, the yield of NK0238 was higher than the yields obtained with 1.50 or 1.75 equiv. (Table 3, entries 1–3); in addition, the use of 2.00 equiv. of the amine greatly reduced the amount of the main byproduct of this reaction. Next, we optimized the reaction temperature (entries 3–7). We found that the purity of the NK0238 product increased with increasing reaction temperature. Given that there was little difference between the purity at 50 °C and that at 60 °C (entries 6 and 7, respectively), 50 °C was chosen as the optimal temperature (94% yield, nearly 97% HPLC purity).

Given that the main impurity in the reaction product was excess *n*-octylamine, we developed a method for eliminating the amine during the post-reaction treatment of NK0238 when we performed a scale-up experiment (150 mmol) under the optimal conditions. We took advantage of the difference between the solubilities of the salts of *n*-octylamine and NK0238 in ethyl acetate. Specifically, we added 1.1 equiv. of HCl in ethyl acetate to form *n*-octylamine hydrochloride, which precipitated from the reaction mixture and was filtered off (24.0 g, recovery rate 97%). The filtrate was then treated with excess HCl in ethyl acetate to generate NK0238 hydrochloride, which was collected by filtration (48.6 g, 138 mmol). Neutralization with saturated aqueous sodium carbonate to give NK0238 (43.0 g, 136 mmol) at 91% yield and 98% HPLC purity (Fig. 7).

3.2 Phytotoxicity

To study whether NK0238 has side effects on test plants, the phytotoxicity of NK0238 was tested. The results showed that NK0238 was safe for test plants at 100 mg·L^{-1}.

3.3 Antiviral activities in the greenhouse

First, antivirus spectrum of NK0238 was studied. The bioactivity results show that it provided useful inactivation of many plant viruses. The inactivation of SCMV (sugarcane mosaic virus), PMMoV (pepper mild mottle virus) and TMV by NK0238 was similar to or higher than that of ningnanmycin at 100 mg·L^{-1}

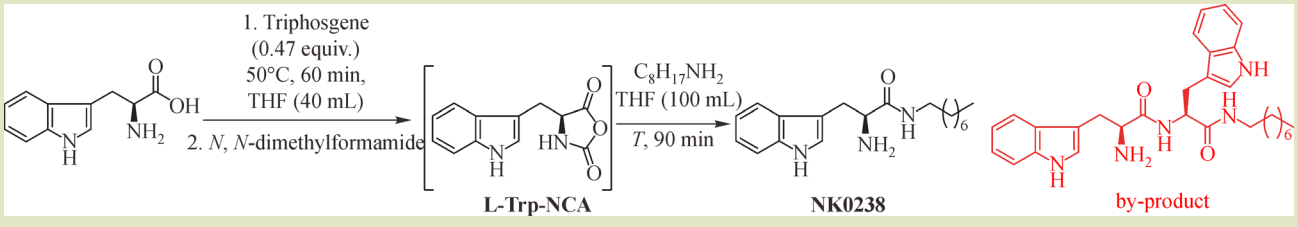


Fig. 6 Optimization of reaction conditions for the second step.

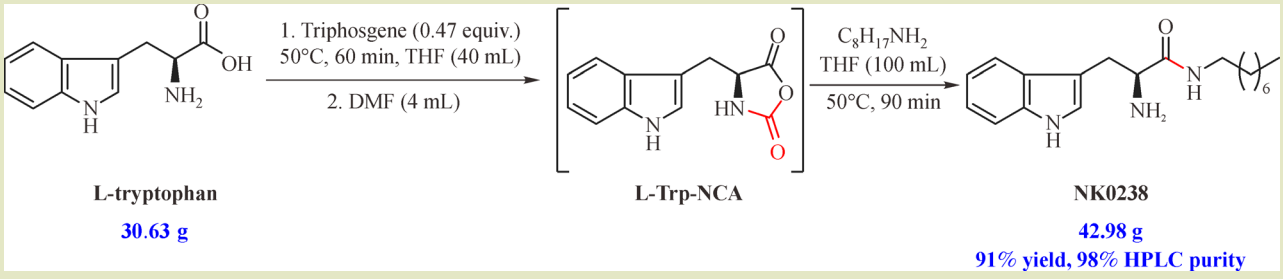


Fig. 7 Scaled-up synthesis of the anti-plant-virus candidate, NK0238.

(Table 4). In addition, NK0238 exhibited higher protective and curative effects against TMV in pepper (*Capsicum*) than ningnanmycin at 100 mg·L^{-1} (Table 5). We also studied the control of TMV by NK0238 when applied by spray at different stages (Table 6). The results showed that NK0238 has useful anti-TMV activity in tobacco at the seedling stage (NK0238 applied before virus inoculation), in seedlings recovering from virus infection (virus inoculated before NK0238 application) and the rosette stage (virus inoculated before NK0238 application), which was similar to ningnanmycin. The anti-TMV effect of NK0238 was best when applied before virus inoculation during the seedling stage.

3.4 Field experiments

The field experiments with NK0238 applied against TMV, panax notoginseng virus Y, gladiolus mosaic virus, banana bunchy top virus were equal to or higher than with amino-oligosaccharins and moroxydine hydrochloride-copper acetate (Table 7). NK0238 (100 g·ha^{-1}) showed higher activity against TMV than with amino-oligosaccharin (100 g·ha^{-1}) and morpholine hydrochloride-copper acetate (600 g·ha^{-1}). The control effect of NK0238 (10 g·ha^{-1}) against panax notoginseng virus Y was significantly higher than with amino-oligosaccharins (100 g·ha^{-1}) and moroxydine hydrochloride-copper acetate (600 g·ha^{-1}). NK0238 (10 g·ha^{-1}) exhibited higher activity against gladiolus mosaic virus than morpholine hydrochloride-copper acetate (600 g·ha^{-1}). The control effect of NK0238 (100 g·ha^{-1}) against banana bunchy top virus was higher than with amino-oligosaccharin (100 g·ha^{-1}) and morpholine hydrochloride-copper acetate (600 g·ha^{-1}).

3.5 Toxicity

To compare the toxicity versus efficacy of NK0238, we conducted toxicity tests. The results of ecotoxicological testing

Table 4 Inactivation effect against plant viruses, SCMV, PMMoV and TMV[a]

Compound	Concentration (mg·L^{-1})	Inactivation effect (%)		
NK0238	100	69±1[b]	78±2[c]	55±3[d]
Ningnanmycin	100	31±2[b]	74±2[c]	50±1[d]

Note: [a]SCMV, sugarcane mosaic virus (maize used as test plant); PMMoV, pepper mild mottle virus (pepper used as test plant); TMV, tobacco mosaic virus (tobacco K326 used as test plant). [b]Inactivation effect against SCMV. [c]Inactivation effect against PMMoV. [d]Inactivation effect against TMV.

Table 5 Protective effect and curative effect against plant virus, TMV[a]

Compound	Concentration (mg·L^{-1})	Control effect (%)	
NK0238	100	50±1[b]	55±3[c]
Ningnanmycin	100	44±1[b]	50±1[c]

Note: [a]Pepper was used as test plant. [b]Protective effect. [c]Curative effect.

Table 6 Anti-TMV effects at different treatment dates[a]

Compound	Concentration (mg·L^{-1})	Control effect (%)		
NK0238	100	72±2[b]	62±1[c]	49±3[d]
Ningnanmycin	100	67±1[b]	61±2[c]	50±1[d]

Note: [a]Pepper was used as a test plant. [b]Spraying at seedling stage, inoculating virus 3 days later. [c]Inoculating virus at seedling stage, then spraying after the seedling recovering from transmitting. [d]Inoculating virus at seedling stage, then spraying at rosette stage.

Table 7 Field experiments

Compound	Concentration (gai·L^{-1})	Average control effect 10 days after the third time spraying			
NK0238	10	46±1[a]	55±2[b]	52±1[c]	38±1[d]
Amino-oligosaccharins (5% aqueous solution)	100	65±1[a]	40±1[b]	66±3[c]	52±1[d]
Moroxydine hydrochloride-cupric acetate (20% WP)	600	66±1[a]	42±3[b]	42±1[c]	38±1[d]

Note: [a]Control effect against TMV. [b]Control effect against Panax notoginseng virus Y. [c]Control effect against Gladiolus mosaic virus. [d]Control effect against Banana bunchy top virus.

show that NK0238 was not harmful to birds, fish, bees and silkworms (Table 8).

4 CONCLUSIONS

In summary, we developed a two-step synthetic route for the antiviral plant protectant, NK0238. By this route, NK0238 can be obtained at 94% yield and nearly 97% HPLC purity by reaction of *n*-octylamine with crude L-trp-NCA, which is synthesized from L-tryptophan and triphosgene. Compared with the previously reported routes, this route has the advantages of high atom economy, high yield and operational simplicity. In addition, it can be used for the preparation of more than 40 g of NK0238 in a single batch. After completing the process optimization, we conducted an in-depth study of antiviral activity in greenhouse and field experiments and toxicity tests. NK0238 exhibited a broad antiviral spectrum, in field experiments, the activities of NK0238 against TMV, panax notoginseng virus Y, gladiolus mosaic virus, banana bunchy top virus were equal to or higher than amino-oligosaccharins and moroxydine hydrochloride-copper acetate. The results of ecotoxicological testing showed that the compound was not harmful to birds, fish, bees and silkworms. This compound has an excellent prospects for commercial development.

Table 8 Ecotoxicology assessment of the anti-plant-virus candidate, NK0238

Bird (*Coturnix japonica*)		Fish (*Danio rerio*)		Honey bee (*Apis mellifera*)		Silkworm (*Bombyx mori*)	
LD_{50} (mg·kg^{-1} bw)	Toxicity	LC_{50} (mg·L^{-1})	Toxicity	LD_{50} (μg per bee)	Toxicity	LC_{50} (mg·L^{-1})	Toxicity
> 2000	low	> 10	low	> 100	low	> 1	–

Supplementary materials

The online version of this article at https://doi.org/10.15302/J-FASE-2021390 contains supplementary materials. Mechanisms of reactions of tryptophan with phosgene and triphosgene (Fig. S1); optimization of conditions for for synthesis of L-NAC from phosgene (Table S1); screening of quenchers for synthesis of L-NAC from phosgene (Table S2); ^{1}H and ^{13}C NMR Spectra of L-trp-NCA, NK0238, and byproduct (Figs. S2–S7); HPLC standard curve for NK0238 (Fig. S8); HPLC spectrum of NK0238 in scaled-up reaction (Fig. S9); Chiral chromatographic separation (Table S3, Fig. S10–S11); the inactivation, protective and curative effect against TMV (Fig. S12); and procedures for the antivirus and field trial are provided in the supplementary materials.

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Compliance with ethics guidelines

Wentao Xu, Hao Tian, Hongjian Song, Yuxiu Liu, Yongqiang Li, and Qingmin Wang declare that they have no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by any of the authors.

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