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Design, Synthesis, and Insecticidal Activities of Novel Pyranoside Derivatives Targeting at Potential Second Calcium Channel IP_3 Receptor

Changchun Wu, Yuxin Li, Ming Liu, Jingbo Liu, Xuewen Hua, Dongkai Zhang, Lixia Xiong, Na Yang, Baolei Wang, and Zhengming Li*

State Key Laboratory of Elemento-Organic Chemistry, *Collaborative Innovation Center of Chemical Science and Engineering* (*Tianjin*), *Nankai University*, *Tianjin 300071*, *China*

In order to search for potent and environmental friendly insecticides with new modes of action, a series of pyranoside derivatives mimicking D-*myo*-inositol 1,4,5-trisphosphate (IP_3) were designed and synthesized according to the bioisosteric approach. The biological assay indicated that most of the new compounds showed moderate to good insecticidal activities against oriental armyworm (*Mythimna separata*) and diamondback moth (*Plutella xylostella*). Especially, compound **5g** displayed 80% larvicidal activity against oriental armyworm at 50 mg/L. Meanwhile, **5a** showed 100% and 70% larvicidal activities against diamondback moth at 50 and 25 mg/L, respectively. To further explore the mode of action, calcium imaging technique was applied to study the effects of **5a**, **5g**, and **5i** on the intracellular calcium ion concentration ($[Ca^{2+}]_i$) in central neurons isolated from *Spodoptera exigua*. The results indicated that the tested compounds released stored calcium ions from endoplasmic reticulum.

Keywords pyranoside derivatives, insecticidal activity, IP3, bioisosteric approach

Introduction

In recent years, some conventional insecticides such as methamidophos, dicofol, monocrotophos, fipronil, *etc*. have been prohibited in China due to their toxicity to mammals or damage to the proliferation of bees. Therefore, the discovery of new potent insecticides with novel modes of action is crucial to overcoming the resistance and ecological problems. Anthranilic diamides were discovered by DuPont as a new class of insecticides targeting at the insect ryanodine receptors (RyRs).[1,2] This category of insecticides has the intrinsic selectivity for the insect RyRs without any unfavorable effects towards mammals.[3] They can evoke massive calcium release from intracellular stores, disrupt calcium homeostasis, and cause the final death of insects.[4,5]

Ca^{2+} release is mediated by intracellular ligand-gated calcium release channels, which have been identified as the ryanodine receptors (RyRs) and inositol 1,4,5-trisphosphate receptors (IP_3Rs).[6] There are functional similarities between RyRs and IP_3Rs channels.[7,8] Chlorantraniliprole and Cyantraniliprole (Figure 1) have been marketed as environmentally-benign and highly potent activators of insect RyRs. However, to the best of our knowledge, little attention has been paid to discovering new insecticides which target at the insect IP_3Rs.

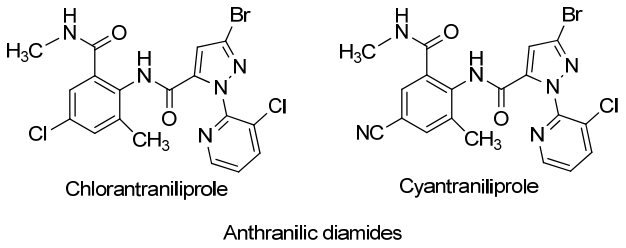

Figure 1 Chemical structures of chlorantraniliprole and cyantraniliprole.

D-myo-Inositol 1,4,5-trisphosphate (IP_3, Figure 2) is an intracellular second messenger that stimulates mobilization of intracellular Ca^{2+} stores.[9] When IP_3 interacts with a site on the N-terminal portion of its receptor, Ca^{2+} ions are released from intracellular stores to the cytosol.[10,11] IP_3 analogues have shown diverse biological activities, such as anticancer and anti-inflammatory activities in mammals study.[12-14] Adenophostins A and B, as penicillium fermentated natural products, were reported to stimulate the release of Ca^{2+} from IP_3-sensitive intracellular stores.[15,16] The adenophostins were reported as pharmacological tools to investigate cell signalling mechanisms of mammals.[17,18] They

* E-mail: nkzml@vip.163.com; Tel.: 0086-022-23503732; Fax: 0086-022-23503732
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were shown to be 10- to 100-fold more potent than IP_3 in releasing Ca^{2+} and in receptor binding assays.[19] A crystal structure of the IP_3-binding core of mouse IP_3R1 in complex with IP_3 was preliminarily confirmed by X-ray technique,[10] and the delicate structure of insect IP_3Rs still remains unknown. Till today, there are no reports on the study of new IP_3Rs modulators of any insects.

Considering a bioisosteric and mimicking approach, inositol connected to a phosphate moiety in IP_3 could be replaced by a six member ring pyranoside. Inspired by commercial RyRs insecticides, we first reported here the design and synthesis of pyranoside derivatives containing phosphorus moieties as potential IP_3Rs modulators. To explore its mode of action, the effect of target compounds on $[Ca^{2+}]_i$ in the central neurons isolated from *S. exigua* were studied by calcium imaging technique.[20]

Experimental

General

^1H NMR, ^{13}C NMR and ^{31}P NMR spectra were recorded on a Bruker AV 400 spectrometer in $CDCl_3$ solution with tetramethylsilane as the internal standard. High-resolution mass spectrometry (HRMS) data were recorded on an Agilent 6520 Q-TOF LC/MS spectrometer with ESI resource. The reagents used were all analytically or chemically pure. All solvents were dried by standard methods in advance and distilled before use. All yields referred to isolated yields and were not optimized. Spots were visualized by spraying with phosphomolybdic acid (10% in EtOH) followed by heating or by dipping into an aqueous basic solution of $KMnO_4$ followed by heating. *D-myo*-Inositol 1,4,5-trisphosphate hexasodium salt (IP_3Na) was purchased from J&K Scientific Ltd (Beijing, China).

Synthesis of allyl 2,6-di-*O*-trimethylacetyl-3,4-bis-*O*-(diethoxyphosphoryl)-α-*D*-glucopyranoside (5a)

A solution of *n*-BuLi (1.9 mL, 4.8 mmol, 2.5 mol/L in hexane) was added to a stirred mixture of allyl 2,6-di-*O*-trimethylacetyl-α-*D*-glucopyranoside **1** (0.78 g, 2 mmol) in dry THF (15 mL) at −78 ℃ under nitrogen. After reacting for 30 min at −78 ℃, diethyl chlorophosphate (1.04 g, 6 mmol) was added. The solution was then warmed to room temperature gradually and stirred overnight. The reaction was quenched with water (20 mL), followed by extraction with EtOAc (30 mL × 3). The combined organic layers were washed with saturated $NaHCO_3$ (50 mL), brine (50 mL), and dried over $MgSO_4$. After filtration and evaporation *in vacuo*, the obtained yellow crude residue was purified by column chromatography on silica gel using petroleum ether/EtOAc as eluent (V/V=3∶1 then 1∶1) to afford **5a** as a colorless liquid.

Synthesis of allyl 2,6-di-*O*-trimethylacetyl-3,4-bis-*O*-(diphenoxyphosphoryl)-α-*D*-glucopyranoside (5b)

Intermediate **1** (1.16 g, 3 mmol) was dissolved in anhydrous THF (20 mL) under nitrogen and cooled to −78 ℃. *n*-Butyllithium (2.9 mL, 7.2 mmol, 2.5 mol/L in hexane) was then added, followed by addition of diphenyl chlorophosphate (1.1g, 9 mmol). After stirring for 25 min at −78 ℃, the solution was warmed to room temperature overnight. After completion of the reaction, the mixture was poured into a suspension of diethyl ether (60 mL) and water (60 mL), and extracted with EtOAc (90 mL). The combined organic layers were washed with saturated $NaHCO_3$ (60 mL) and dried with anhydrous $MgSO_4$. The mixture was concentrated *in vacuo* and purified by column chromatography on silica gel using petroleum ether/EtOAc as eluent [V/V=10∶1 with 2% Et_3N] to afford **5b** (1.15 g, 45%) as a yellow liquid.

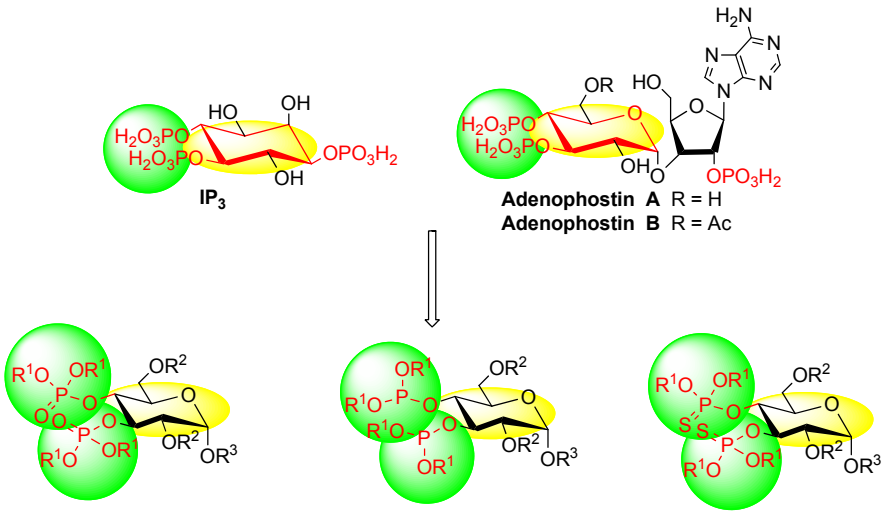


Figure 2 Chemical structures of some representative compounds and design of the title compounds.

Synthesis of 1-*O*-allyl-2,6-di-*O*-trimethylacetyl-*α*-*D*-glucopyranosyl-3,4-bis-(dibenzylphosphite) (5c)

1*H*-Tetrazole (1.12 g, 16 mmol) was added portionwise to a stirred solution of **1** (1.56 g, 4 mmol) in dry CH_2Cl_2 (30 mL) at room temperature under nitrogen. Dibenzyl-*N*,*N*-diisopropylphosphoramidite (4 mL, 12 mmol) was then added. After stirring at room temperature overnight, the solution was concentrated *in vacuo*. The residue was purified by column chromatography on silica gel [petroleum ether/EtOAc (*V* ∶ *V*＝50 ∶ 1) with 2% Et_3N] to yield **5c** as a colorless oil.

Synthesis of allyl 2,6-di-*O*-trimethylacetyl-3,4-bis-*O*-(dibenzyloxyphosphoryl)-*α*-*D*-glucopyranoside (5d)

To a stirred solution of **1** (1.16 g, 3 mmol) and 1*H*-tetrazole (0.84 g, 12 mmol) in dry CH_2Cl_2 (15 mL) at room temperature under nitrogen was added dibenzyl-*N*,*N*-diisopropylphosphoramidite (3 mL, 9 mmol). After reacting for 12 h, the mixture was then cooled to −78 ℃, followed by addition of *m*-CPBA (2.43 g, 85%, 12 mmol) in portions over 10 min. The resulting mixture was warmed to room temperature gradually, and stirred overnight. The reaction was quenched with a 10% aqueous solution of Na_2SO_3 (25 mL). The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with saturated $NaHCO_3$ (120 mL), dried over $MgSO_4$, and concentrated. Purification of the residue by column chromatography on silica gel [petroleum ether/EtOAc 6 ∶ 1 then 3 ∶ 1 (*V* ∶ *V*)] gave **5d** as a yellow liquid.

Synthesis of allyl 2,6-di-*O*-trimethylacetyl-3,4-bis-*O*-(dibenzyloxythiophosphoryl)-*α*-*D*-glucopyranoside (5e)

Compound **1** (0.78 g, 2 mmol) was dissolved in dry CH_2Cl_2 (25 mL) under nitrogen, and 1*H*-tetrazole (0.42 g, 6 mmol) was added, followed by dibenzyl-*N*,*N*-diisopropylphosphoramidite (1.9 mL, 5 mmol). After stirring for 6 h at room temperature, a solution of phenylacetyl disulfide (3.0 g, 10 mmol) in CH_2Cl_2 was added. The resulting mixture was stirred for another 24 h. The solvent was removed under reduced pressure, and the residue was partitioned with ethyl acetate (60 mL) and water (60 mL). The organic layer was washed with brine, dried, and concentrated. The residue was purified by column chromatography on silica gel [(petroleum ether/EtOAc 50 ∶ 1 then 20 ∶ 1 (*V* ∶ *V*)] to afford **5e** as a yellow liquid.

Synthesis of 1-*O*-allyl-2,6-di-*O*-trimethylacetyl-*α*-*D*-glucopyranosyl-3,4-bis-(diethylphosphite) (5f)

Intermediate **1** (1.56 g, 4 mmol) and diisopropylethylamine (2.58 g, 20 mmol) were dissolved in dry CH_2Cl_2 (40 mL) under nitrogen atmosphere. The resulting mixture was cooled to −40 ℃, and diethyl chlorophosphite (1.88 g, 12 mmol) was added dropwise. After stirring for 30 min at −40 ℃, the solution was warmed to room temperature and stirred overnight. The reaction was quenched with crushed ice. The organic layer was washed with brine (30 mL), dried over $MgSO_4$, and filtrated. Evaporation of the filtrate *in vacuo* gave the yellow residue, which was purified by column chromatography on silica gel [petroleum ether/EtOAc 60 ∶ 1 (*V* ∶ *V*) with 2% Et_3N] to afford pure **5f** as a colorless oil.

Synthesis of allyl 2,6-di-*O*-trimethylacetyl-3,4-bis-*O*-(diethoxythiophosphoryl)-α-*D*-glucopyranoside (5g)

To a mixture of **1** (1.16 g, 3 mmol) and diisopropylethylamine (1.94 g, 15 mmol) in dry CH_2Cl_2 (40 mL) at −40 ℃ under nitrogen was added diethyl chlorophosphite (1.41 g, 9 mmol) dropwise. After reacting for 1 h at −40 ℃, the mixture was warmed to room temperature slowly and stirred for another 12 h. Then, a solution of phenylacetyl disulfide (3.0 g, 10 mmol) in CH_2Cl_2 was added. After 24 h, the mixture was diluted with EtOAc (90 mL), washed with water. The organic layer was dried over $MgSO_4$, and concentrated under reduced pressure to give the yellow residue, which was purified by silica gel column chromatography [petroleum ether/EtOAc 50 ∶ 1 then 20 ∶ 1 (*V* ∶ *V*)] to give **5g** as a yellow liquid.

Synthesis of allyl 2,6-di-*O*-benzyl-3,4-bis-*O*-(diethoxyphosphoryl)-*α*-*D*-glucopyranoside (5h)

The same procedure was followed as that described above for title compound **5a**, except for the use of allyl 2,6-di-*O*-benzyl-*α*-*D*-glucopyranoside **4** instead of intermediate **1**. All other steps remained the same.

Synthesis of *n*-propyl 3,4-bis-*O*-(diethoxyphosphoryl)-*α*-*D*-glucopyranoside (5i)

To a solution of compound **5h** (0.5 g, 0.8 mmol) in methanol was added Pd-C (10%, 1.0 g) at room temperature. The reaction was stirred under an atmospheric pressure of hydrogen for 24 h. The resulting mixture was then filtered through a pad of Celite. Evaporation of the filtrate followed by column chromatography on silica gel [petroleum ether/EtOAc 10 ∶ 1 then 2 ∶ 1 (*V* ∶ *V*)] gave **5i** as a colorless liquid.

Synthesis of allyl 2,6-di-*O*-benzyl-3,4-bis-*O*-(diethoxythiophosphoryl)-*α*-*D*-glucopyranoside (5j)

The same procedure was used as that described above for title compound **5g**, except for the use of **4** as the substrate instead of **1**. All other steps remained the same.

Biological assay

Insecticidal activities against oriental armyworm (*Mythimna separata*) and diamondback moth (*Plutella xylostella*) were performed on test organisms reared in a greenhouse. The bioassay was replicated at (25±1) ℃ according to statistical requirements. Assessments were made on a dead/alive basis, and mortality rates were corrected using Abbott's formula. The error of the experiments was 5%. For comparative purposes, D-*myo*-

inositol 1,4,5-trisphosphate hexasodium salt (IP_3Na) was tested as positive control under the same conditions.

Larvicidal activity against oriental armyworm

The larvicidal activities of compounds **5a**—**5j** and D-*myo*-inositol 1,4,5-trisphosphate hexasodium salt were evaluated according to the leaf-dip method.[21] Leaf disks were cut from fresh corn leaves and then dipped into the test solution. After drying, the treated leaf disks were placed individually into a glass-surface vessel. Then, 10 third-instar oriental armyworm larvae were put into each vessel containing dried treated leaf disk. The symptoms of affected larvae were observed 1 d after treatment. Mortality rates were evaluated 3 d after treatment. Each treatment was replicated three times.

Larvicidal activity against diamondback moth

The larvicidal activities of compounds **5a**—**5j** and *D*-*myo*-inositol 1,4,5-trisphosphate hexasodium salt were tested by the leaf dip method.[22] At first, a solution of each test sample in DMF (AR, purchased from Alfa Aesar) at a concentration of 1000 mg/L was prepared and then diluted to the required concentration with distilled water. Leaf disks were cut from fresh cabbage leaves and then dipped into the test solution for 10 s. After drying, the resulting leaf disks were placed individually into glass tubes. Each disk was infested with 30 third-instar diamondback moth larvae. Percentage mortalities were evaluated 3 d after treatment. Each treatment was performed three times.

Calcium imaging

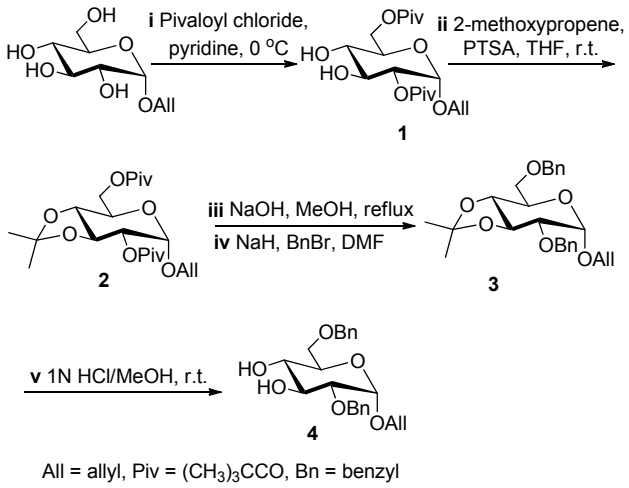
Calibration of the fluorescence signal was achieved by using the method of Takahashi *et al*. with modifications.[23] Briefly, the attached neurons were rinsed in standard physiological saline [(mmol/L): NaCl 150, KCl 4, $MgCl_2$ 2, $CaCl_2$ 2, HEPES 10, buffered to pH 7.0] and then incubated in the dark for 45 min at 28 ℃ in standard external saline containing the dye fluo-3 AM (10 μmol/L). After dye loading, cells were again rinsed in physiological saline twice. Calcium free extracellular fluid had the following composition (mmol/L): NaCl 150, KCl 4, $MgCl_2$ 2, EGTA 2, Hepes 10, buffered to pH 7.0. After dye loading, neurons were again rinsed in standard physiological saline twice. Calcium ratio imaging studies were conducted using the imaging system coupled to an inverted fluorescence microscope with a Fluor 40× oil immersion objective (Olympus IX71). Cells were excited at 488 nm and the 530 nm fluorescence emission acquired using a CCD (Image Pro-6.0).

Results and Discussion

Chemistry

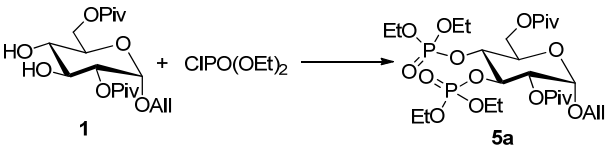
The key intermediates allyl 2,6-di-*O*-trimethylacetyl-*α*-*D*-glucopyranoside (**1**) and allyl 2,6-di-*O*-benzyl-*α*-*D*-glucopyranoside (**4**) were synthesized referring to the previous reports as shown in Scheme 1.[24,25]

Scheme 1 Synthesis of intermediates **1** and **4**



With the diol intermediate **1** in hand, we focused our attention on the preparation of the glycoside bisphosphate **5a** (Table 1). Initially, **1** was treated with diethyl chlorophosphate in the presence of K_2CO_3 or KOH in acetone under reflux. However, the expected product **5a** was not detected (Entries 1—2). Then, **1** was reacted with diethyl chlorophosphate in the presence of DMAP at room temperature.[26] To our delight, the desired product was isolated in 10% yield (Entry 3). In order to increase the yield, the reaction was conducted in CH_2Cl_2 under reflux. To our disappointment, elevating the reaction temperature merely led to some side rections (Entry 4). It was found that the exposure of **1** to *n*-BuLi/diethyl chlorophosphate resulted in the formation of product **5a** in 65% yield (Entry 5).[27]

Table 1 Optimization of the reaction conditions



Entry	Reaction condition	Yield[a]/%
1	K_2CO_3/acetone/reflux	no reaction
2	KOH/acetone/reflux	no reaction
3	DMAP/CH_2Cl_2/r.t./48 h	10
4	DMAP/CH_2Cl_2/reflux/12 h	side reaction
5	*n*-Buli/THF/−78 ℃ to r.t.	65

[a] Isolated yield.

As illustrated in Scheme 2, **1** was subjected to the optimized conditions (*n*-BuLi, THF, −78 ℃ to r.t.) with diphenyl chlorophosphate to give **5b** in 45% yield. The reaction of **1** with dibenzyl-*N*,*N*-diisopropylphosphoramidite and 1*H*-tetrazole in CH_2Cl_2 furnished bis-

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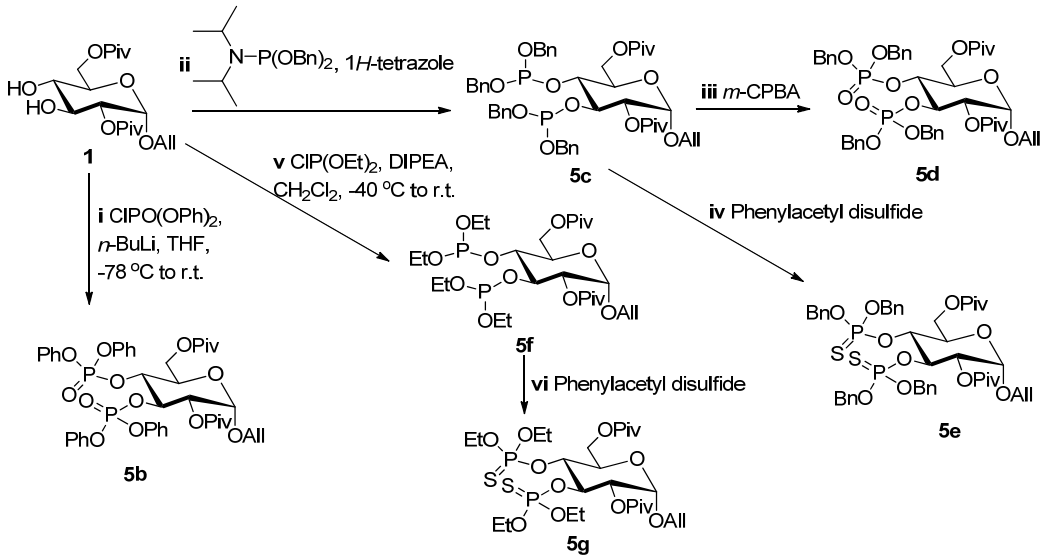
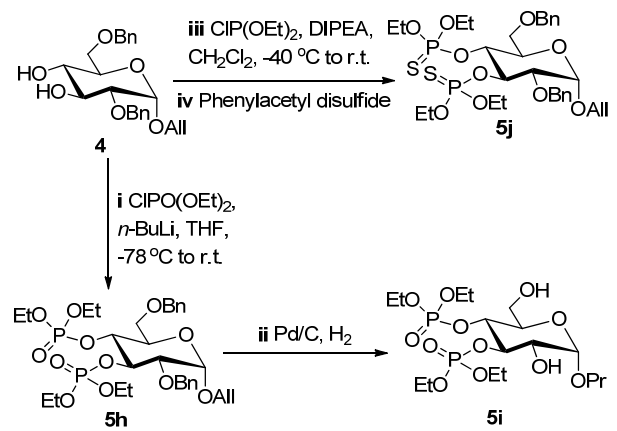
Scheme 2 Synthetic route of target compounds **5b**—**5g**



phosphites **5c**, which could be stored without decomposition in a freezer (at −20 ℃) under nitrogen. **5c** was then oxidized with *m*-CPBA to give **5d** in 32% overall yield (2 steps).[28] Unfortunately, the desired oxidative product was not obtained when sulphur (S_8) was used as the oxidant.[29] Further treatment of **5c** with phenylacetyl disulfide in CH_2Cl_2 provided the expected bisphosphorothioates **5e**. On the other hand, **1** reacted with diethyl chlorophosphite in the presence of DIPEA to give **5f** in 50% yield.[30] Product **5f** should be also stored in a freezer under nitrogen. Thionation of **5f** with phenylacetyl disulfide in CH_2Cl_2 at room temperature gave **5g** in 48% overall yield (2 steps).

Intermediate **4** was then smoothly converted to **5h** by exposure to *n*-BuLi/diethyl chlorophosphate in 52% yield (Scheme 3). Further reaction of **5h** with Pd/C under a pressure of H_2 provided **5i** in 84% yield. Finally, **4** was treated with diethyl chlorophosphite in the presence of DIPEA providing the corresponding bisphosphites, which further underwent a one-pot thionation with phenylacetyl disulfide to give **5j** in 47% yield.

Scheme 3 Synthetic route of target compounds **5h**—**5j**



Biological activities

Larvicidal activity against oriental armyworm The insecticidal activities of title compounds and IP_3 against oriental armyworm were listed in Table 2, from which we observed that IP_3 displayed a 50% fatal rate at 200 mg/L. Furthermore, **5a** and **5j** showed 20% insecticidal activity at 100 mg/L (200 mg/L, 100%). It was worth noting that **5g** showed excellent larvicidal activities even at 50 mg/L, which was far better than that of IP_3.

Table 2 Insecticidal activities of compounds **5a**—**5j** and IP_3 against oriental armyworm

Compd.	Larvicidal activity/% at conc./(mg•L^{-1})			
	500	200	100	50
5a	100	100	**20**	0
5b	50	15	0	0
5c	60	15	0	0
5d	60	30	0	0
5e	30	10	0	0
5f	75	50	0	0
5g	100	100	100	**80**
5h	60	40	0	0
5i	60	20	0	0
5j	100	100	**20**	0
IP_3Na	NT[a]	50	0	0

[a] NT: not tested

The bioassay results indicated that the substituted groups had a significant influence on the larvicidal activities. Compared with **5a**, **5b** and **5d**, it was observed that the sequence of larvicidal activity was Et＞Bn＞Ph in the phosphate moiety. The bioassay results of **5e** and

5g also followed the above sequence. It was concluded that bisphosphites **5c** and **5f** showed lower larvicidal activities than bisphosphates **5d** and **5a**, respectively. In addition, bisthiophosphates **5g** and **5j** showed higher larvicidal activities than bisphosphates **5a** and **5h**. The bioactivities of compounds **5a**, **5h**, and **5g**, **5j** indicated that the sequence of larvicidal activity was trimethylacetyl＞benzyl in the different substituents at the C-2 and C-6 positions of the pyranoside ring. The hydroxyl groups at the C-2 and C-6 positions of the pyranoside bisphosphate **5i** did not have a positive effect on the larvicidal activities. Hence, it was concluded that the trimethylacetyl and diethyoxylthiophosphoryl groups connected to oxygen atoms on the pyranoside ring had a significant effect on the larvicidal activities and were key pharmacophores.

Figure 3 showed the biological symptoms of larvaes affected by title compound **5g**, IP_3 and chlorantraniliprole (Rynaxypyr) individually. Insects treated with **5g** and IP_3 showed typical Ca^{2+} ion efflux symptoms such as insect body contraction, body shortening and feeding cessation. The symptoms were similar to those of larvaes treated with commercial Rynaxypyr.

Figure 3 Symptoms of third-instar larvae of oriental armyworm treated by leaf dipping. (a) **5g**, (b) IP_3, (c) Rynaxypyr, (d) Untreated.

Larvicidal activity against diamondback moth Table 3 showed the insecticidal activity of compounds **5a**－**5j** and IP_3 against diamondback moth. On the whole, most of the tested compounds displayed good to excellent larvicidal activities at 50 mg/L. Particularly, **5a** and **5c** possessed 100% fatal rate at 50 mg/L, a little higher than that of IP_3Na (80%, 50 mg/L). It was noted that **5a**, **5c**, and **5i** exhibited moderate larvicidal activities at 25 mg/L. Additionally, compounds **5a**－**5j** and IP_3Na showed low insecticidal activity at 10 mg/L. For example, the larvicidal activities of **5a** and IP_3Na at 10 mg/L were 40% and 45%, respectively.

The bioactivities of **5a**, **5b**, and **5d** revealed that the sequence of larvicidal activity was Et＞Ph＞Bn in the phosphate moiety. However, the sequence of insecticidal activity was Bn＞Et in the phosphite and thiophosphate moieties (**5c**＞**5f**, **5e**＞**5g**). Moreover, bisphosphites **5c** and **5f** were more effective than the corresponding bisthiophosphates **5e** and **5g**, respectively. From Table 3, we could also conclude that the substituents (such as pivaloyl, Bn, H) in the various moieties at the C-2 and C-6 positions of pyranoside ring had minimal impact on biological activities. In general, the structures of the title compounds had no significant effect on the larcidical activity against diamondback moth.

Table 3 Insecticidal activities of compounds **5a**－**5j** and IP_3 against diamondback moth

Compd.	Larvicidal activity/% at conc./(mg•L^{-1})					
	500	200	100	50	25	10
5a	100	100	100	**100**	**70**	**40**
5b	100	100	100	80	40	0
5c	100	100	100	100	**55**	30
5d	100	100	60	0	0	0
5e	100	100	100	70	20	0
5f	100	100	100	85	45	20
5g	100	100	100	70	0	0
5h	100	100	100	80	20	0
5i	100	100	100	90	**50**	0
5j	100	100	100	85	40	0
IP_3Na	100	100	100	**80**	**60**	**45**

Electrophysiological recordings

To determine whether the title compounds could be involved in calcium signaling transduction and the target of the novel compounds, we examine the effects on the central neurons of *S. exigua* on the calcium homeostasis with calcium imaging technique after the neurons loading with fluo-3 AM.

Figure 4 showed the change of $[Ca^{2+}]_i$ vs. recording time when the neurons were treated with **5a**, **5i**, **5g** and chlorantraniliprole in the absence of extracellular calcium. Application of compound **5** could increase the cytosolic calcium concentration. The peaks of $[Ca^{2+}]_i$ were elevated to (104.2±1.37)% (n = 6), (104.7±2.1)% (n = 6), (106.2±2.7)% (n = 6) and (111.8±3.5)% (n=6) of the initial value after treatments of the neurons with high concentration of **5a**, **5i**, **5g** and chlorantraniliprole, respectively.

Figure 5 showed the change of $[Ca^{2+}]_i$ vs. recording time when the neurons were treated with **5g** (100 mg•L^{-1}), IP_3Na (500 mg•L^{-1}) and chlorantraniliprole (1 mg•L^{-1}) in the absence of extracellular calcium. The experiment results showed that lower chlorantraniliprole could increase the free calcium and compound **5g** could not. On the other hand, after the neurons were treated with IP_3Na for 2.5 min, the peaks of $[Ca^{2+}]_i$ were elevated to (112.2±3.10)% (n=6) and two peaks were

observed through the recording. Although this novel compound could cause the claium to be elevated in the neurons, the concentration was too high.

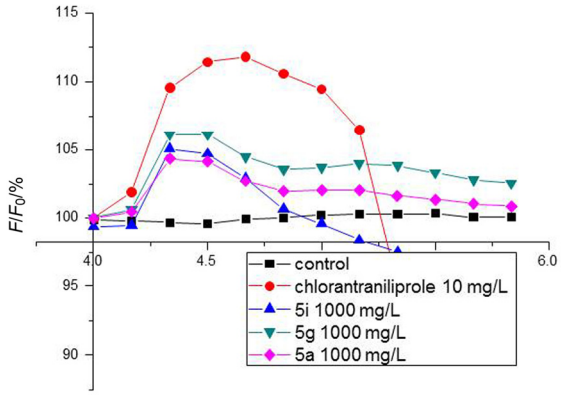


Figure 4 The change of $[Ca^{2+}]_i$ versus recording time when the neurons were treated with **5a**, **5g**, **5i** and chlorantraniliprole. The central neurons of *S. exigua* third larvae were dyed by loading with fluo-3 AM.

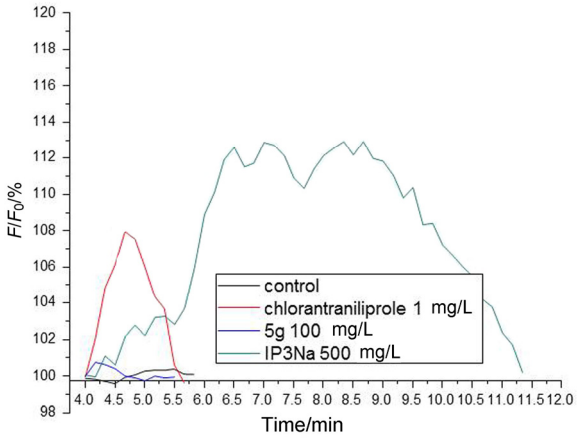


Figure 5 The change of $[Ca^{2+}]_i$ versus recording time when the neurons were treated with **5g**, IP_3Na and chlorantraniliprole. The central neurons of *S. exigua* third larvae were dyed by loading with fluo-3 AM.

Conclusions

In summary, ten novel compounds were designed, synthesized and evaluated for their insecticidal activities against oriental armyworm and diamondback moth. The structures were characterized by ^1H NMR, ^{13}C NMR, ^{31}P NMR, and HRMS. The bioassays showed that most compounds exhibited good insecticidal activities. In particular, compound **5g** had better larvicidal effects against oriental armyworm than IP_3. Moreover, compound **5a** exhibited the same larvicidal level as IP_3 against diamondback moth. The symptoms of larvaes treated with **5g** and IP_3 were similar to those of larvaes affected by chlorantraniliprole. The calcium imaging technique demonstrated that the tested compounds targeted at the calcium release channel on the endoplasmic reticulum. The tested compounds could be considered to be a new lead of potential IP_3Rs modulators. Further structural optimization is currently undertaken in our laboratory.

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(Zhao, X.)

