JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Synthesis of a Series of Monosaccharide−Fipronil Conjugates and Their Phloem Mobility

Jian-Guo Yuan,[†,‡,§] Han-Xiang Wu,[†,‡,§] Meng-Ling Lu,[†,‡] Gao-Peng Song,[†,‡] and Han-Hong Xu*[,†,‡]

[†]State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, and [‡]Key Laboratory of Natural Pesticide and Chemical Biology, Ministry of Education, South China Agricultural University, Guangzhou, Guangdong 510642, People's Republic of China

ABSTRACT: To test the effect of adding different monosaccharide groups to a non-phloem-mobile insecticide on the phloem mobility of the insecticide, a series of conjugates of different monosaccharides and fipronil were synthesized using the trichloroacetimidate method. Phloem mobility tests in castor bean (*Ricinus communis* L.) seedlings indicated that the phloem mobility of these conjugates varied markedly. L-Rhamnose−fipronil and D-fucose−fipronil displayed the highest phloem mobility among all of the tested conjugates. Conjugating hexose, pentose, or deoxysugar to fipronil through an O-glycosidic linkage can confer phloem mobility to fipronil in *R. communis* L. effectively, while the −OH orientation of the monosaccharide substantially affected the phloem mobility of the conjugates.

KEYWORDS: *Fipronil, monosaccharide, phloem transport*

INTRODUCTION

To apply pesticides to control pest efficiently, phloem-mobile pesticides need to be developed.[1−3] A strategy for developing phloem-mobile pesticides is to add an acidic group to a non-systemic pesticide. For instance, a conjugate of oxamyl with glucuronic acid exhibits good phloem mobility.[4] Another efficient strategy is to conjugate an endogenous phloem mobile substrate, such as an amino acid or carbohydrate, to the active non-phloem-mobile pesticide molecules to make them phloem-mobile using the plant endogenous transporters. Several derivatives of pesticides with an α-amino acid have been reported. ε-(2,4-Dichlorophenoxyacetic acid)-L-lysine (2,4-D-Lys), which is one of these derivatives, exhibits good phloem mobility, and its uptake is mediated by an active carrier system.[5−7] Glucosylation and glutathionation have been proven important for vacuolar uptake of sulfonylurea herbicides.[8]

In recent studies,[9,10] we synthesized a novel conjugate of the insecticide fipronil, *N*-[3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1*H*-pyrazol-5-yl]-1-(β-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-methanamine (GTF) (Figure 1), which contains a glucose moiety. GTF exhibits moderate phloem mobility, and the phloem transport property of fipronil has not yet been reported.[11,12] We showed that conjugating glucose to fipronil could confer phloem mobility to fipronil using plant monosaccharide transporters.

The monosaccharide transporter gene superfamily consists of many members, and many of these proteins are capable of transporting more than one monosaccharide. For example, AtSTP1, which is one of the *Arabidopsis* sugar transporters, transports glucose but is also capable of transporting galactose, mannose, and xylose.[13,14] Therefore, we have been inspired to research phloem mobility of the conjugates of fipronil with different monosaccharides. Furthermore, although the linkage of GTF is a N-glycosidic bond, O-glycosidic bonds are the most common linkages found in natural glycosides. Herein, we report the synthesis of a series of monosaccharide−fipronil conjugates that link with an O-glycosidic bond and describe their phloem mobility in *Ricinus communis* L. seedlings.

MATERIALS AND METHODS

General Information for Synthesis. Solvents were used as purchased without further purification. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AV-600 instrument. Chemical shifts were expressed in parts per million, with tetramethylsilane (TMS) as the internal standard. The mass spectra (MS) of new compounds were obtained by MAT 95 (Thermo) with electron impact (EI) ionization or Bruker maXis 4G ESI−Q-TOF mass spectrometer. Analytical thin-layer chromatography (TLC) was performed on silica gel GF254. Silica gel was used for column chromatography.

Plant Materials. Castor bean seeds (*R. communis* L.) were obtained from the Agricultural Science Academy of Zibo Shandong China and grown as previously described.[15] Seedlings were grown in moist vermiculite for 7−8 days, and average-sized seedlings were selected for the experiments.

Insects. Larvae of *Spodoptera litura* F. were obtained from the Guangzhou Biological Control Station and raised with artificial feed at 26 ± 2 °C and 60% relative humidity under a photoperiod of 16:8 h (light/dark).

Membrane Potential Measurements. Plasma membrane potential was measured according to our previously described measurements.[9] The fluorescent membrane potential indicator dye, bis-(1,3-dibutylbarbituric acid)-trimethine oxonol [$DiBAC_4(3)$], was selected as the fluorescent membrane potential indicator dye. The method was established using flow cytometric analysis of the protoplasts of *R. communis* cotyledons. The protoplasts were suspended in buffer solution without (control) or with title compounds at 100 μM concentration for 2 h, and then the protoplast suspension was incubated with 2 μM $DiBAC_4(3)$ for 30 min at 28 °C prior to flow cytometric analysis.

Phloem Sap Collection and Analysis. The phloem sap collection method was similar to that recently described.[9] The cotyledons were

Received: February 25, 2013
Revised: April 14, 2013
Accepted: April 15, 2013
Published: April 15, 2013

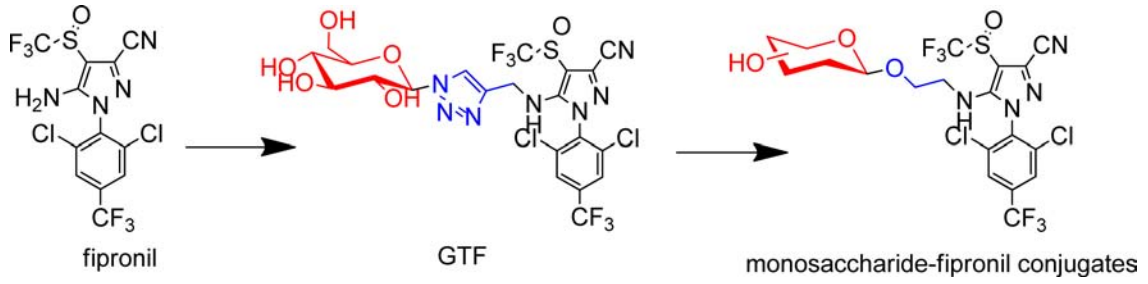


Figure 1. Chemical structure of fipronil, GTF, and monosaccharide−fipronil conjugates.

incubated in buffered solution containing 20 mM 2-(*N*-morpholino)-ethanesulfonic acid (MES), 0.25 mM $MgCl_2$, and 0.5 mM $CaCl_2$ at pH 5.5, and each chemical was used at 100 μM in these experiments. The phloem sap was analyzed by high-performance liquid chromatography (HPLC) after dilution with water (phloem sap/water, 1:5, v/v). Agilent Technologies 1100 HPLC system with a vacuum degasser, a quaternary pump, an autosampler, and an ultraviolet−visible (UV−vis) detector were employed for analysis. An Agilent C18 reversed-phase column (5 μm, 250 × 4.6 mm inner diameter) was used and maintained at 30 °C. The mobile phase consisted of acetonitrile and water (50:50, v/v), at a flow rate of 1 mL min^{-1}, and the injection volume was 10 μL. The absorbance wavelength was 210 nm. An external calibration method was used to quantify the title compounds. A series of standard solutions of title compounds (0.5, 1, 5, 10, 25, 50, and 100 μM) for linearities were prepared in methanol. The linear equations and limits of detection (LODs) of the title compounds were shown in Table 1. The LODs were calculated as a signal/noise ratio = 3.

Table 1. Linear Equations, Correlation Coefficients, and LODs of the HPLC Method for Quantification of Compounds 4a−4j

compound	linear equation	correlation coefficient	LOD (mg/L)
4a	$y = 20.08x + 7.53$	0.9994	0.04
4b	$y = 20.17x + 6.05$	0.9995	0.06
4c	$y = 20.92x - 0.69$	0.9997	0.02
4d	$y = 20.11x - 7.23$	0.9997	0.09
4e	$y = 20.99x + 4.08$	0.9991	0.07
4f	$y = 20.82x - 2.95$	0.9998	0.06
4g	$y = 20.15x - 1.88$	0.9997	0.07
4h	$y = 20.98x - 0.39$	0.9996	0.04
4i	$y = 20.28x - 0.72$	0.9995	0.05
4j	$y = 20.77x - 2.55$	0.9997	0.05

*Synthesis of Ethyl N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-aminoacetate (**1**) (Scheme 1).* In an ice bath, sodium hydride (0.96 g, 40 mmol) was added in a portion to the solution of fipronil (4.37 g, 10 mmol) in dry tetrahydrofuran (THF, 40 mL), and then ethyl bromoacetate (1.11 mL, 10 mmol) was added dropwise. The mixture was then stirred at room temperature for 3 h.

The reaction mixture was quenched by adding ice water, and the resultant mixture was extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed with aqueous sodium hydrogen carbonate and brine, dried with sodium sulfate, filtered, and evaporated *in vacuo*. The residues were purified by column chromatography to obtain the desired product **1** as a yellow solid in 91% yield.

^{1}H NMR ($CDCl_3$) δ: 7.85−7.79 (m, 2H), 6.05 (t, J = 5.2 Hz, 1H), 4.21−4.16 (m, 2H), 3.81−3.76 (m, 1H), 3.74−3.68 (m, 1H), 1.28−1.23 (m, 3H). ^{13}C NMR ($CDCl_3$) δ: 168.2, 151.1, 136.8, 136.7, 135.6, 135.5, 135.4, 128.7, 127.3, 126.6, 126.5 (two), 124.6, 124.3, 122.8, 122.1, 121.0, 119.2, 110.3, 96.0, 62.7, 45.9, 14.1. EI−MS, m/z 522 M^+.

*Synthesis of N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)-phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-aminoethanol (**2**) (Scheme 1).* To a stirred solution of compound **1** (0.523 g, 1 mmol) in THF (10 mL) was added $NaBH_4$ (0.072 g, 2 mmol) and $ZnCl_2$(0.136g, 1mmol). The reaction mixture was heated at 50 °C using a preheated oil bath and stirred for 1 h. After completion, the pH value of the reaction mixture was adjusted to 7−8 by 1.0 M HCl, and the resultant mixture was extracted with ethyl acetate (15 mL × 3). The combined organic layers were washed with aqueous sodium hydrogen carbonate and brine, dried with sodium sulfate, filtered, and evaporated *in vacuo*. The residues were purified by column chromatography to obtain the desired product **2** as a yellow solid in 56% yield.

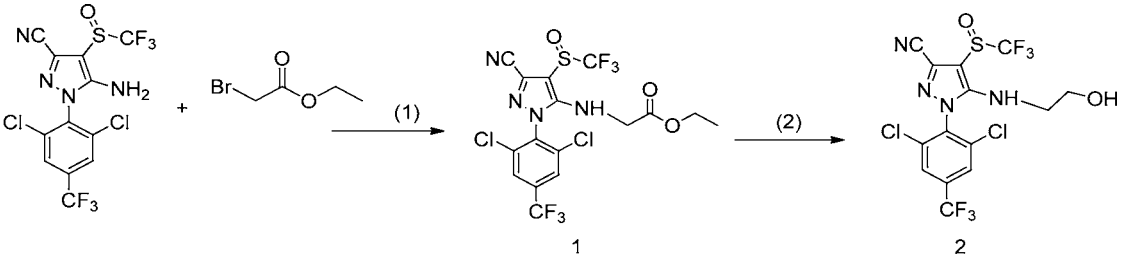
^{1}H NMR (DMSO-d_6) δ: 8.36 (s, 1H), 8.34 (d, J = 1.2 Hz, 1H), 7.59 (t, J = 6.0 Hz, 1H), 4.97 (s, 1H), 3.56−3.47 (m, 2H), 3.27−3.18 (m, 2H). ^{13}C NMR (DMSO-d_6) δ: 150.7, 136.0, 135.9, 134.6, 133.8, 133.6, 133.4, 128.9, 127.0, 126.9, 126.7, 126.6, 125.6, 124.4, 123.2, 121.4, 111.6, 99.6, 94.7, 60.1, 46.6. EI−MS, m/z 480 M^+.

General Procedure for Synthesis Title Compounds (4a−4j). Trichloroacetimidate donors (**a**−**j**) (Scheme 2) were synthesized as previously described.[16,17]

Step 1 (Scheme 3): A solution of trichloroacetimidate donor (**a**−**j**) (1 mmol) and compound **2** (1 mmol) in anhydrous CH_2Cl_2 (10 mL) was stirred at room temperature with activated 4 Å molecular sieves for 30 min. The reaction mixture was then cooled to −30 °C, and BF_3·OEt_2 (0.5 mmol) was added dropwise. The reaction was carried out under an atmosphere of nitrogen. After stirring at −30 °C for 1 h and then at room temperature for 0.5 h, the reaction mixture was quenched with Et_3N and filtered through celite. The filtrate was concentrated and purified by flash chromatography to afford compounds **3a**−**3j** as solids.

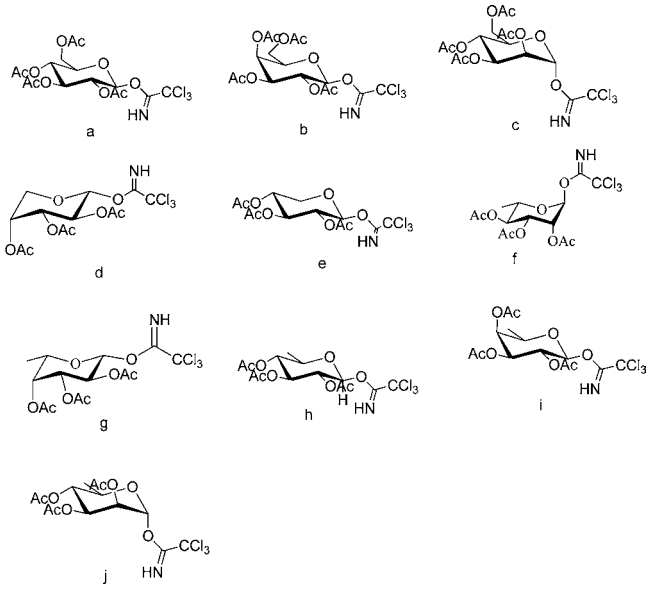
Step 2 (Scheme 3): The solid above (0.5 mmol) was added to a solution of sodium methoxide in dry methanol (0.05 M, 5 mL). The resultant solution was stirred for 30 min at room temperature. The

Scheme 1. Reagents and Conditions: (1) NaH, THF, and 2 h and (2) $NaBH_4$, $ZnCl_2$, THF, 50 °C, and 1 h



dx.doi.org/10.1021/jf400888c | J. Agric. Food Chem. 2013, 61, 4236−4241

Scheme 2. Monosaccharide Trichloroacetimidate Donors



mixture was neutralized with Amberlite IR120 (H^+) resin and filtered, and the filtrate was then evaporated. The residues were purified by column chromatography to obtain the desired products **4a**–**4j** (Figure 2).

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl β-D-Glucopyranoside (**4a**).* Yield of 62% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1:1. ^{1}H NMR (CD_3OD) δ: 8.11–8.08 (m, 2H), 4.26 (d, J = 7.7 Hz, 1H), 4.25* (d, J = 7.7 Hz, 1H), 4.06–4.01 (m, 1H), 3.86 (t, J = 11.6 Hz, 1H), 3.85* (t, J = 11.6 Hz, 1H), 3.75–3.71 (m, 1H), 3.70–3.66* (m, 1H), 3.63–3.56 (m, 2H), 3.52–3.48 (m, 1H), 3.45–3.41 (m, 1H), 3.37–3.22 (m, 2H), 3.19 (t, J = 9.2 Hz, 1H), 3.19* (t, J = 9.2 Hz, 1H) (* represents another diastereoisomer). ^{13}C NMR (CD_3OD) δ: 152.5, 152.4, 138.0 (two), 137.9 (two), 136.3, 136.2, 136.1, 135.9, 130.3, 128.1, 128.0, 127.9, 127.8, 127.7, 125.9, 124.6, 122.8, 120.9, 112.3, 104.3, 104.1, 96.8, 78.1, 78.0, 77.9, 75.1, 75.0, 71.7, 71.6, 69.4, 69.3, 62.9, 62.8, 46.3, 45.7. HRMS (EI) calcd for $C_{20}H_{18}Cl_2F_6N_4O_7S$, 642.0172; found, 642.0169.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl β-D-Galactopyranoside (**4b**).* Yield of 57% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1:1. ^{1}H NMR (CD_3OD) δ: 8.10–8.06 (m, 2H), 4.21 (d, J = 7.7 Hz, 1H), 4.20* (d, J = 7.7 Hz, 1H), 4.06–4.01 (m, 1H), 3.81–3.79 (m, 1H), 3.73–3.66 (m, 3H), 3.60–3.55 (m, 1H), 3.50–3.40 (m, 4H). ^{13}C NMR (CD_3OD) δ: 152.5, 152.4, 138.1, 138.0, 137.9, 136.3, 136.2, 136.1 (two), 135.9 (two), 130.3, 128.1, 128.0, 127.9, 127.8, 126.4, 125.9, 124.6, 122.8, 112.3, 104.9, 104.7, 96.8, 76.8, 76.7, 75.0, 74.8, 72.4, 70.3, 70.2, 69.4, 69.2, 62.5 (two), 46.4, 45.8. HRMS (ESI) calcd for $C_{20}H_{18}Cl_2F_6N_4NaO_7S$ [M + Na]$^+$, 665.0075; found, 665.0073.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl α-D-Mannopyranoside (**4c**).* Yield of 52% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1.3:1. ^{1}H NMR (CD_3OD) δ: 8.12–8.07 (m, 2H), 4.77 (d, J = 1.3 Hz, 1H), 4.71* (d, J = 1.2 Hz, 1H), 3.95–3.85 (m, 1H), 3.84–3.80 (m, 1H), 3.79–3.74

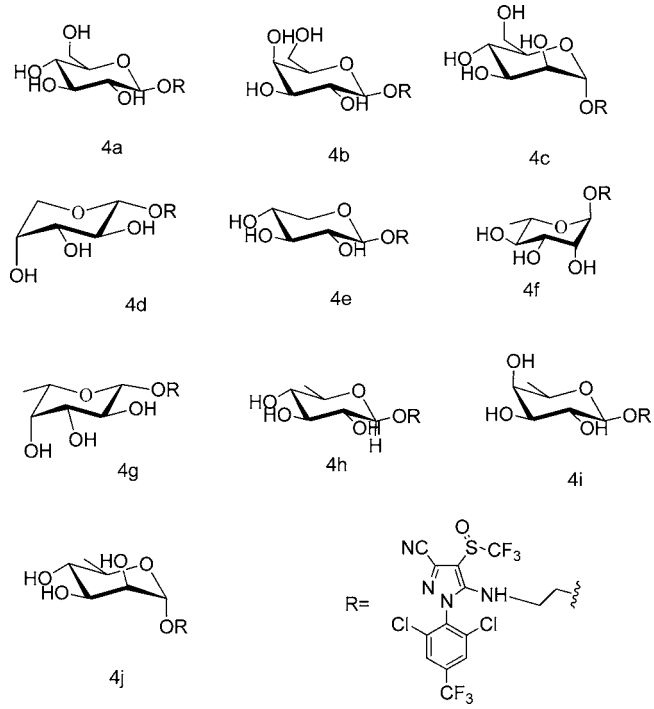
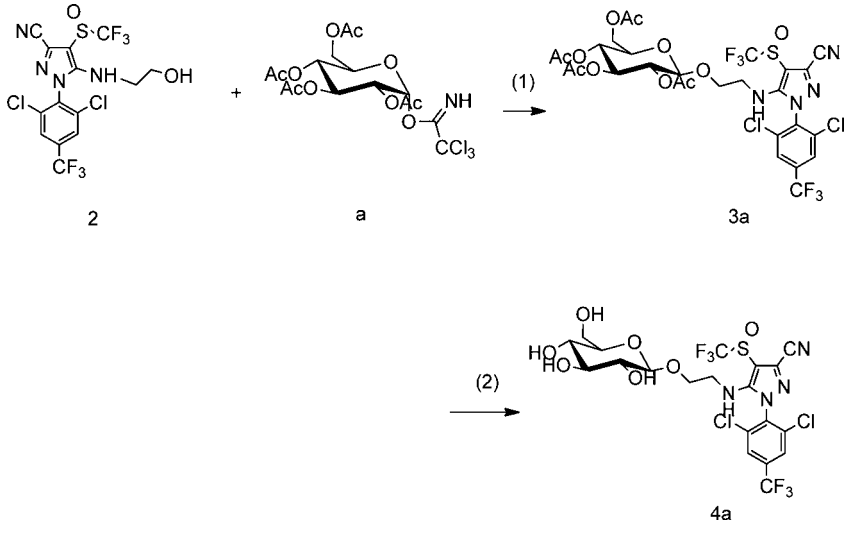


Figure 2. Structures of designed monosaccharide–fipronil conjugates. The monosaccharides of these conjugates fall within the following three categories: hexose (**4a**–**4c**), pentose (**4d** and **4e**), and deoxysugar (**4f**–**4j**).

Scheme 3. Reagents and Conditions: (1) $BF_3{\cdot}OEt_2$, CH_2Cl_2, and 4 Å molecular sieves and (2) CH_3OH and CH_3ONa



dx.doi.org/10.1021/jf400888c | J. Agric. Food Chem. 2013, 61, 4236–4241

(m, 1H), 3.63 (dd, J = 6.4, 11.6 Hz, 1H), 3.60−3.55 (m, 1H), 3.56−3.47 (m, 4H), 3.46−3.41 (m, 1H). ^{13}C NMR (CD_3OD) δ: 152.4, 152.3, 138.1, 138.0, 137.9, 137.8, 136.2 (two), 136.0 (two), 128.2, 128.0, 127.8 (two), 126.0, 124.6, 122.8, 112.4, 102.7, 102.1, 96.4, 74.9, 74.7, 72.5, 72.4, 71.9, 71.8, 69.1, 68.6, 68.5, 68.2, 62.9, 45.5. HRMS (ESI) calcd for $C_{20}H_{18}Cl_2F_6N_4NaO_7S$ $[M + Na]^+$, 665.0075; found, 665.0071.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl α-D-Arabopyranoside (**4d**).* Yield of 56% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1:1. ^{1}H NMR (CD_3OD) δ: 8.10−8.07 (m, 2H), 4.18 (d, J = 6.6 Hz, 1H), 4.17* (d, J = 6.6 Hz, 1H), 3.94−3.88 (m, 1H), 3.81−3.77 (m, 2H), 3.72−3.68 (m, 1H), 3.66−3.62* (m, 1H), 3.55−3.40 (m, 5H). ^{13}C NMR (CD_3OD) δ: 152.4, 138.1, 137.9 (two), 136.3, 136.1 (two), 128.1, 128.0, 127.9, 127.8 (two), 126.4, 125.9, 124.6, 123.7, 122.7, 120.9, 112.3, 104.9, 104.8, 97.7, 74.2, 74.1, 72.3, 69.5, 69.2 (two), 66.9 (two), 46.3, 45.9. HRMS (EI) calcd for $C_{19}H_{16}Cl_2F_6N_4O_6S$, 612.0066; found, 612.0066.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl β-D-Xylopyranoside (**4e**).* Yield of 45% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1:1.2. ^{1}H NMR (CD_3OD) δ: 8.10−8.07 (m, 2H), 4.19 (d, J = 7.6 Hz, 1H), 4.18* (d, J = 7.6 Hz, 1H), 3.93−3.89 (m, 1H), 3.80−3.72 (m, 2H), 3.69−3.64 (m, 1H), 3.60−3.56* (m, 1H), 3.48−3.36 (m, 2H), 3.28 (dd, J = 9.0, 1.6 Hz, 1H), 3.26* (dd, J = 9.0, 1.6 Hz, 1H), 3.17−3.09 (m, 2H). ^{13}C NMR (CD_3OD) δ: 152.5, 152.3, 138.0, 137.9 (two), 136.2, 136.2, 136.1, 130.3, 128.1, 128.0, 127.9, 127.8, 126.4, 125.9, 124.5, 123.7, 120.9, 112.3, 105.1, 104.9, 96.9, 77.8, 77.7, 74.9, 74.7, 71.1, 71.0, 69.6, 69.3, 66.9, 46.2, 45.7. HRMS (EI) calcd for $C_{19}H_{16}Cl_2F_6N_4O_6S$, 612.0066; found, 612.0064.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl α-L-Rhamnopyranoside (**4f**).* Yield of 51% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1:1. ^{1}H NMR (CD_3OD) δ: 8.12−8.08 (m, 2H), 4.69 (d, J = 1.4 Hz, 1H), 4.64* (d, J = 1.4 Hz, 1H), 3.87 (dd, J = 3.4, 1.4 Hz, 1H), 3.82−3.74 (m, 1H), 3.73−3.68 (m, 1H), 3.58−3.48 (m, 3H), 3.39−3.43 (m, 1H), 3.37 (t, J = 9.4 Hz, 1H), 3.36* (t, J = 9.4 Hz, 1H), 1.26 (d, J = 6.1 Hz, 3H), 1.24* (d, J = 6.1 Hz, 3H). ^{13}C NMR (CD_3OD) δ: 152.4, 152.3, 138.0 (two), 137.9, 137.8, 136.2, 136.0, 130.4, 128.2, 128.0, 127.9, 127.8, 126.4, 126.0, 124.6, 123.8, 122.7, 120.9, 112.4, 102.8, 102.2, 96.6, 73.8, 73.7, 72.3, 72.2, 72.1, 71.9, 70.1, 69.9, 69.0, 68.1, 45.5, 45.4, 18.0 (two). HRMS (EI) calcd for $C_{20}H_{18}Cl_2F_6N_4O_6S$, 626.0223; found, 626.0220.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl β-L-Fucopyranoside (**4g**).* Yield of 39% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1.1:1. ^{1}H NMR (CD_3OD) δ: 8.09−8.06 (m, 2H), 4.18 (d, J = 7.2 Hz, 1H), 3.99−3.93 (m, 1H), 3.72−3.63 (m, 1H), 3.62−3.55 (m, 2H), 3.53−3.46 (m, 1H), 3.45−3.38 (m, 3H), 1.24 (d, J = 5.3 Hz, 3H), 1.23* (d, J = 5.3 Hz, 3H). ^{13}C NMR (CD_3OD) δ: 149.6 (two), 135.2, 135.1, 135.0, 133.4, 133.3, 133.0, 127.5, 127.4, 125.2, 125.0, 123.0, 121.7, 119.9, 109.4 (two), 101.8, 101.7, 94.0 (two), 76.6, 72.3, 72.1, 70.1, 70.0, 69.3, 69.2, 69.1, 43.4, 43.0, 13.8 (two). HRMS (ESI) calcd for $C_{20}H_{19}Cl_2F_6N_4O_6S$ $[M + H]^+$, 627.0307; found, 627.0301.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl β-D-6-Deoxyglucopyranoside (**4h**).* Yield of 53% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1.1:1. ^{1}H NMR (CD_3OD) δ: 8.10−8.07 (m, 2H), 4.22 (d, J = 9.6 Hz, 1H), 4.21* (d, J = 9.6 Hz, 1H), 4.00−3.93 (m, 1H), 3.73−3.69 (m, 1H), 3.60−3.65 (m, 1H), 3.50−3.45 (m, 1H), 3.29−3.24 (m, 2H), 3.18−3.09 (m, 1H), 2.99−2.93 (m, 1H), 1.24 (d, J = 6.2 Hz, 3H), 1.23* (d, J = 6.2 Hz, 3H). ^{13}C NMR (CD_3OD) δ: 152.6, 152.4, 137.9 (two), 136.2 (two), 135.9, 128.1, 127.9, 127.8, 127.7, 125.9, 124.6, 122.8, 112.3 (two), 104.2, 104.0, 102.9, 96.9, 79.5, 77.8, 77.6, 76.9, 75.3, 75.2, 73.4, 73.3, 66.7, 69.2, 46.2, 45.4, 18.1, 18.0. HRMS (ESI) calcd for $C_{20}H_{19}Cl_2F_6N_4O_6S$ $[M + H]^+$, 627.0307; found, 627.0303.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl β-D-Fucopyranoside (**4i**).* Yield of 41% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1.1:1. ^{1}H NMR (CD_3OD) δ: 8.09−8.05 (m, 2H), 4.18 (d, J = 7.2 Hz, 1H), 3.99−3.93 (m, 1H), 3.72−3.63 (m, 1H), 3.62−3.59 (m, 1H), 3.58−3.54 (m, 2H), 3.52−3.46 (m, 1H), 3.43−3.38 (m, 2H), 1.25−1.22 (m, 3H). ^{13}C NMR (CD_3OD) δ: 152.5 (two), 138.1, 138.0, 137.9 (two), 136.2, 136.1 (two), 135.9 (two), 128.0 (two), 127.9, 127.8, 125.9, 124.5, 122.8, 112.3, 104.8, 104.6, 102.9, 73.0, 72.9, 72.2 (two), 72.0 (two), 69.3, 69.2, 46.3, 45.9, 16.7 (two). HRMS (ESI) calcd for $C_{20}H_{19}Cl_2F_6N_4O_6S$ $[M + H]^+$, 627.0307; found, 627.0303.

*N-[3-Cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1H-pyrazol-5-yl]-2-aminoethyl α-D-Rhamnopyranoside (**4j**).* Yield of 49% for two steps, white solid, and the product was isolated as a pair of diastereoisomers in a ratio of 1.4:1. ^{1}H NMR (CD_3OD) δ: 8.12−8.08 (m, 2H), 4.69 (d, J = 1.2 Hz, 1H), 4.64* (d, J = 1.2 Hz, 1H), 3.90−3.86 (m, 1H), 3.80−3.75 (m, 1H), 3.73−3.68 (m, 1H), 3.58−3.50 (m, 3H), 3.44−3.49 (m, 1H), 3.35 (t, J = 9.5 Hz, 1H), 1.25 (d, J = 6.3 Hz, 3H), 1.23* (d, J = 6.1 Hz, 3H). ^{13}C NMR (CD_3OD) δ: 152.4, 152.3, 138.0 (two), 137.9, 137.8, 136.2, 136.1, 136.0, 128.2, 128.0 (two), 127.9, 127.8, 126.0, 124.6, 122.8, 112.4, 102.8, 102.2, 96.6, 96.5, 79.5, 73.8, 73.7, 72.3, 72.2, 72.1, 71.9, 70.1, 69.9, 69.0, 68.1, 45.4, 45.3, 18.0 (two). HRMS (ESI) calcd for $C_{20}H_{18}Cl_2F_6N_4NaO_6S$ $[M + Na]^+$, 649.0126; found, 649.0123.

*Insecticidal Activity of Compounds **4a**−**4j** against S. litura F.* Assessments of compounds **4a**−**4j** and fipronil bioactivities on the third instar larvae of *S. litura* F. were according to the previously described procedure[9,18] by the leaf disk-dipping assay.

Test compounds were dissolved in acetone and suspended in distilled water containing Tween 80 (0.1%), and the concentration of acetone is below 5%. Leaf disks were dipped in each test solutions for 30 s, after air-drying, the treated leaf disks were placed into Petri dishes (9 cm in diameter). A total of 10 third-instar larvae of *S. litura* were released into each dish. Distilled water containing acetone (5%) and Tween 80 (0.1%) solutions was used as the control. Petri dishes were incubated at 26 ± 2 °C and 60% relative humidity under a photoperiod of 16:8 (light/dark). All treatments were repeated 3 times. Mortalities were observed 48 h later.

RESULTS AND DISCUSSION

Synthesis and Characterization. To create a glycosidic linkage between fipronil and the monosaccharide, we used the trichloroacetimidate method.[19−21] In glycosylation reactions, glycosyl donors possess an acyloxyl group with a participating function at C_2, which exclusively yields the corresponding 1,2-*trans* glycoside with quite high stereoselectivity.[21] Finally, a series of conjugates of different monosaccharides and fipronil with 1,2-*trans* O-glycosidic linkage (**4a**−**4j**) were synthesized.

The structures of compounds **4a**−**4j** were confirmed via ^{1}H NMR, ^{13}C NMR, and mass spectrometry. The ^{1}H NMR spectra of compounds **4a**−**4j** showed the presence of a pair of diastereoisomers,[22] and from these spectra, we could determine the ratio of these diastereoisomers. For example, the ^{1}H NMR spectrum of compound **4a** showed two doublets at 4.26 and 4.25 ppm with J = 7.7 Hz, indicating both the presence of diastereoisomers and the β configuration of the glucosidic linkage.

Phloem Mobility. To evaluate the phloem mobility of compounds **4a**−**4j**, we used the castor bean system.[15,23,24] As previously reported,[9] the plasma membrane potential with compounds **4a**−**4j** treatments was measured by flow cytometric analysis of the protoplasts of *R. communis* cotyledons. In comparison to the control, the relative fluorescence data were not significantly different during the treatment period (Figure 3). The results indicate that compounds **4a**−**4j** have no depolarizing effects on the transmembrane potential difference (PD) and the xenobiotics tested are not phytotoxic in these short-term

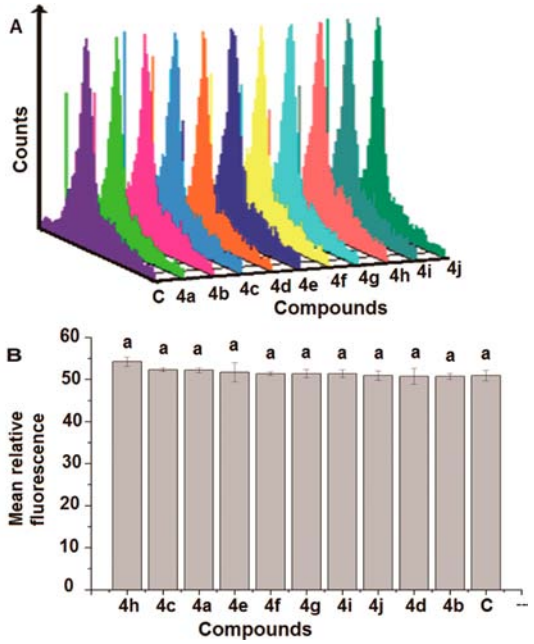


Figure 3. Plasma membrane potential in the protoplasts of *R. communis* cotyledons after 2 h of treatment. (A) Fluorescent intensity histogram marked $DiBAC_4(3)$ of the treatment of compounds **4a**−**4j** and the control (C). (B) Relative fluorescence of the treatment of compounds **4a**−**4j** and the control (C). The protoplasts were suspended in a buffered solution at pH 5.8 with or without (control) compounds **4a**−**4j** (200 μM) for 2 h. The protoplasts were analyzed by flow cytometry for $DiBAC_4(3)$ fluorescence. The data (mean ± SE; $n = 3$) within a column are not significantly different, as shown by the Duncan's multiple range test ($p > 0.05$).

experiments. Therefore, the castor bean system is suitable for testing the phloem systemicity for these conjugates.

The results of the analysis of phloem sap are summarized in Table 2. Among these conjugates, D-fucose−fipronil (**4i**; 27.3 ± 2.1 μM) and L-rhamnose−fipronil (**4f**; 27.3 ± 1.9 μM) exhibited the best phloem mobility, whereas D-galactose−fipronil (**4b**) and D-6-deoxyglucose−fipronil (**4h**) were not detected, thereby indicating that these conjugates did not exhibit phloem mobility.

Table 2. *R. communis* Phloem Sap Analysis after Soaking Cotyledons in a Solution Containing Various Xenobiotics

xenobiotics	incubation medium (μM)	phloem sap[a] (μM)
4i	100	27.3 ± 2.1 a
4f	100	27.3 ± 1.9 a
4a	100	23.0 ± 1.9 ab
4d	100	21.8 ± 1.9 b
4e	100	15.3 ± 2.2 c
4g	100	10.6 ± 1.8 cd
4c	100	7.6 ± 1.6 d
4j	100	6.1 ± 1.4 d
4b	100	ND
4h	100	ND
fipronil	200	ND[b]

[a]Phloem sap collection started 2 h after the beginning of soaking and lasted for 2 h. Mean ± SE; $n = 3$. ND = not detected. Duncan's multiple range tests at a 5% probability level were used to determine statistical differences among treatments. The data in the table are the mean ± SE, and those followed by different letters in the same column are significantly different at the 5% level. [b]Data are from Yang et al.[9]

These results indicate that the phloem mobility of these conjugates varies significantly. Several conjugates exhibit moderate systemicity, and it can be inferred that some plant sugar carriers may translocate xenobiotics much larger than their natural substrate and with a lipophilic moiety. The monosaccharides of these conjugates fall within the following three categories: hexose, pentose, and deoxysugar. Among the hexose conjugates of fipronil, the analysis of phloem sap showed that the concentrations of compounds **4a** and **4c** were 23.0 ± 1.9 and 7.6 ± 1.6 μM, respectively. Furthermore, compound **4b** was not detected, suggesting that the equatorial −OH group of hexose improved phloem mobility. Among the conjugates of pentose and fipronil, compound **4d** (21.8 ± 1.9 μM) exhibited better phloem mobility than compound **4e** (15.3 ± 2.2 μM), suggesting that the 4-axial −OH group of pentose improved phloem mobility. Among the deoxysugar−fipronil conjugates, compounds **4f** (27.3 ± 2.1 μM) and **4i** (27.3 ± 1.9 μM) both exhibited good phloem mobility, and compound **4h** exhibited no phloem mobility, suggesting that the 4-axial −OH or 2-axial −OH groups of deoxysugar improved phloem mobility. In our previous work, fipronil was not detected in phloem sap under the same experimental conditions.[9] These results demonstrate that conjugating hexose, pentose, or deoxysugar to fipronil through O-glycosidic linkage can confer phloem mobility to fipronil in *R. communis* L. effectively and that the −OH orientation of monosaccharide has a significant effect on the phloem mobility of the conjugates.

Insecticidal Activity. The insecticidal activity of test compounds against *S. litura* F. was evaluated over a wide range of concentrations. The LC_{50} (95% confidence limits) of fipronil is 33.49 mg L^{-1} (25.43−44.12 mg L^{-1}). The title compounds (**4a**−**4j**) did not exhibit insecticidal activity against *S. litura* F. at 100 mg L^{-1}. As noted by Hsu et al.,[4] making derivative pesticides phloem-mobile is often a challenge because of the concomitant loss of activity. Adding the monosaccharide group into fipronil enhanced the phloem systemicity but decreased the insecticidal activity. In our previous study,[9] GTF was reconverted into the parent molecule in the plants; however, whether the conjugates synthesized in the present study can be reconverted into the parent molecule in the different plant organs requires further investigation.

In summary, a series of conjugates of fipronil and monosaccharide were synthesized. The phloem-mobility tests in the castor bean system demonstrated that conjugating hexose, pentose, or deoxysugar to fipronil can confer phloem mobility to fipronil in *R. communis* L. effectively and that the −OH orientation of the monosaccharide substantially affected the phloem mobility of the conjugates. Understanding the mechanisms of phloem systemicity of the monosaccharide−fipronil conjugates and whether these conjugates can be reconverted into the parent molecule require further study.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-20-85285127. Fax: +86-20-38604926. E-mail: hhxu@scau.edu.cn.

Author Contributions

[§]Jian-Guo Yuan and Han-Xiang Wu contributed equally to this work.

Funding

Financial support was from the National Natural Science Foundation of China (Grant 31171886) and the Doctor Science

Research Foundation of the Education Ministry of China (Grant 20114404110020).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

GTF, *N*-[3-cyano-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)-sulfinyl]-1*H*-pyrazol-5-yl]-1-(*β*-D-glucopyranosyl)-1*H*-1,2,3-triazole-4-methanamine; $DiBAC_4(3)$, bis-(1,3-dibutylbarbituric acid)-trimethine oxonol

■ REFERENCES

(1) Pimentel, D. Amounts of pesticides reaching target pests: Environmental impacts and ethics. *J. Agric. Environ. Ethics* **1995**, *8*, 17−29.

(2) Lichtner, F. Phloem mobility of crop protection products. *Funct. Plant Biol.* **2000**, *27*, 609−614.

(3) Brueck, E.; Elbert, A.; Fischer, R.; Krueger, S.; Kuehnhold, J.; Klueken, A. M.; Nauen, R.; Niebes, J. F.; Reckmann, U.; Schnorbach, H. J.; Steffens, R.; van Waetermeulen, X. Movento, an innovative ambimobile insecticide for sucking insect pest control in agriculture: Biological profile and field performance. *Crop Prot.* **2009**, *28*, 838−844.

(4) Hsu, F. C.; Sun, K.; Kleier, D. A.; Fielding, M. J. Phloem mobility of xenobiotics VI. A phloem-mobile pro-nematicide based on oxamyl exhibiting root-specific activation in transgenic tobacco. *Pestic. Sci.* **1995**, *44*, 9−19.

(5) Dufaud, A.; Chollet, J. F.; Rudelle, J.; Miginiac, L.; Bonnemain, J. L. Derivatives of pesticides with an *α*-amino acid function: Synthesis and effect on threonine uptake. *Pestic. Sci.* **1994**, *41*, 297−304.

(6) Chollet, J. F.; Deletage, C.; Faucher, M.; Miginiac, L.; Bonnemain, J. L. Synthesis and structure−activity relationships of some pesticides with an *α*-amino acid function. *Biochim. Biophys. Acta, Gen. Subj.* **1997**, *1336*, 331−341.

(7) Deletage-Grandon, C.; Chollet, J. F.; Faucher, M.; Rocher, F.; Komor, E.; Bonnemain, J. L. Carrier-mediated uptake and phloem system of a 350-Dalton chlorinated xenobiotic with an *α*-amino acid function. *Plant Physiol.* **2001**, *125*, 1620−1632.

(8) Bartholomew, D. M.; Van Dyk, D. E.; Lau, S. C.; O'Keefe, D. P.; Rea, P. A.; Viitanen, P. V. Alternate energy-dependent pathways for the vacuolar uptake of glucose and glutathione conjugates. *Plant Physiol.* **2002**, *130*, 1562−1572.

(9) Yang, W.; Wu, H. H.; Xu, H. H.; Hu, A. L.; Lu, M. L. Synthesis of glucose−fipronil conjugate and its phloem mobility. *J. Agric. Food Chem.* **2011**, *59*, 12534−12542.

(10) Wu, H. X.; Yang, W.; Zhang, Z. X.; Huang, T.; Yao, G. K.; Xu, H. H. Uptake and phloem transport of glucose−fipronil conjugate in *Ricinus communis* involve a carrier-mediated mechanism. *J. Agric. Food Chem.* **2012**, *60*, 6088−6094.

(11) Jiang, D. X.; Lu, X. L.; Hu, S.; Zhang, X. B.; Xu, H. H. A new derivative of fipronil: Effect of adding a glycinyl group to the 5-amine of pyrazole on phloem mobility and insecticidal activity. *Pestic. Biochem. Physiol.* **2009**, *95*, 126−130.

(12) Aajoud, A.; Raveton, M.; Aouadi, H.; Tissut, M.; Ravanel, P. Uptake and xylem transport of fipronil in sunflower. *J. Agric. Food Chem.* **2006**, *54*, 5055−5060.

(13) Sauer, N.; Friedlander, K.; Graml-Wicke, U. Primary structure, genomic organization and heterologous expression of a glucose transporter from *Arabidopsis thaliana*. *EMBO J.* **1990**, *9*, 3045−3050.

(14) Buettner, M. The *Arabidopsis* sugar transporter (AtSTP) family: An update. *Plant Biol.* **2010**, *12*, 35−41.

(15) Chollet, J. F.; Rocher, F.; Jousse, C.; Deletage-Grandon, C.; Bashiardes, G.; Bonnemain, J. L. Synthesis and phloem mobility of acidic derivatives of the fungicide fenpiclonil. *Pest Manage. Sci.* **2004**, *60*, 1063−1072.

(16) Su, Y. H.; Xie, J. S.; Wang, Y. G.; Hu, X.; Lin, X. F. Synthesis and antitumor activity of new shikonin glycosides. *Eur. J. Med. Chem.* **2010**, *45*, 2713−2718.

(17) Hou, S. J.; Kovac, P. A convenient synthesis of furanose-free D-fucose per-*O*-acetates and a precursor for anthrose. *Eur. J. Org. Chem.* **2008**, *11*, 1947−1952.

(18) Sawada, Y.; Yanai, T.; Nakagawa, H.; Tsukamoto, Y.; Tamagawa, Y.; Yokoi, S. J.; Yanagi, M.; Toya, T.; Sugizaki, H.; Kato, Y.; Shirakura, H.; Watanabe, T.; Yajima, Y.; Kodama, S.; Masui, A. Synthesis and insecticidal activity of benzoheterocyclic analogues of *N*′-benzoyl-*N*-(*tert*-butyl)benzohydrazide: Part 3. Modification of *N*-*tert*-butylhydrazine moiety. *Pest Manage. Sci.* **2003**, *59*, 49−57.

(19) Schmidt, R. R.; Kinzy, W. Anomeric-oxygen activation for glycoside synthesis: The trichloroacetimidate method. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21−123.

(20) Liu, Q. C.; Wang, P.; Zhang, L.; Guo, T. T.; Lv, G. K.; Li, Y. X. Concise synthesis of two natural triterpenoid saponins, oleanolic acid derivatives isolated from the roots of *Pulsatilla chinensis*. *Carbohydr. Res.* **2009**, *344*, 1276−1281.

(21) Zeng, Y.; Ning, J.; Kong, F. Z. Pure *α*-linked products can be obtained in high yields in glycosylation with glucosyl trichloroacetimidate donors with a C2 ester capable of neighboring group participation. *Tetrahedron Lett.* **2002**, *43*, 3729−3733.

(22) Zhao, Q. Q.; Li, Y. Q.; Xiong, L. X.; Wang, Q. M. Design, synthesis and insecticidal activity of novel phenylpyrazoles containing a 2,2,2-trichloro-1-alkoxyethyl moiety. *J. Agric. Food Chem.* **2010**, *58*, 4992−4998.

(23) Antognoni, F.; Fornale, S.; Grimmer, C.; Komor, E.; Bagni, N. Long-distance translocation of polyamines in phloem and xylem of *Ricinus communis* L. plants. *Planta* **1998**, *204*, 520−527.

(24) Rocher, F.; Chollet, J. F.; Jousse, C.; Bonnemain, J. L. Salicylic acid, an ambimobile molecule exhibiting a high ability to accumulate in the phloem. *Plant Physiol.* **2006**, *141*, 1684−1693.