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Glucose Positions Affect the Phloem Mobility of Glucose−Fipronil Conjugates

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Supporting Information

ABSTRACT: In our previous work, a glucose−fipronil (GTF) conjugate at the C-1 position was synthesized via click chemistry and a glucose moiety converted a non-phloem-mobile insecticide fipronil into a moderately phloem-mobile insecticide. In the present paper, fipronil was introduced into the C-2, C-3, C-4, and C-6 positions of glucose via click chemistry to obtain four new conjugates and to evaluate the effects of the different glucose isomers on phloem mobility. The phloem mobility of the four new synthetic conjugates and GTF was tested using the *Ricinus* seedling system. The results confirmed that conjugation of glucose at different positions has a significant influence on the phloem mobility of GTF conjugates.

KEYWORDS: *phloem mobility, glucosyl conjugates, click chemistry, glycosyl azides, glucose positions*

INTRODUCTION

The phloem is one of the two long-distance transport pathways in plants.[1] The phloem mobility of pesticides is an essential requirement that contributes positively to its efficacy.[2] Pesticides with good phloem mobility can have an efficacious function in controlling hidden and soil-dwelling pests[3] because these pesticides can be distributed along with the nutrients via the phloem to pest-damaged sites.[4] In addition, the agricultural sector needs novel pesticides that have sufficient phloem mobility in plants because of their evolved resistance, environmental regulations, and economic considerations.[5] For modern synthetic insecticides, the most effective mode of transport in plants is via the xylem, and only a few phloem-mobile insecticides are available. An efficient and economical strategy to develop novel systemic pesticides has been mentioned, in which the structure of the parent compounds is modified by adding endogenous carboxylic acid, amino acid, and hexose derivatives.[2,3,6,7] For example, ε-(2,4-dichlorophenoxyacetyl)-L-Lys, a conjugate of an α-amino acid and an auxenic herbicide, exhibits high phloem mobility in broad bean (*Vicia faba*).[7] IPGN, a novel fluorescent conjugate containing glucose, could enter *Ricinus communis* phloem.[8]

Our research team focuses on exploring the possibility of glycosylation to develop new phloem-mobile pesticides. *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) has been synthesized, and a systemicity test demonstrated that the glucose core confers phloem mobility to fipronil.[9] To fully understand the function of the sugar core in the phloem-mobile molecule, a series of monosaccharide−fipronil conjugates were synthesized in our further study. Phloem mobility tests in castor bean seedlings indicated that the hydroxyl of the monosaccharide has a significant effect on the phloem mobility of the conjugates.[10]

Glucosyl derivatives, which are conjugated at different carbons of glucose, have different properties. For example, the new glucosyl dopamine conjugates substituted at C-6 of the sugar were more potent inhibitors of glucose transport compared to C-1- and C-3-substituted derivatives.[11] (7-Nitrobenz-2-oxa-1,3-diazol-4-yl)-amine (NBD) replaced at C-6 of the sugar cannot be phosphorylated by hexokinase, but NBD substituted at C-2 can be metabolized to 2-NBDG-6-phosphate.[12] In addition, several different (C-2, C-3, C-4, and C-6) natural glucosides are found in nature.[13] Therefore, biological properties of the glucosides have a correlation with different positions of glucose.

All of the monosaccharide−fipronil conjugates were linked at C-1 of the monosaccharide,[8,9] which gives rise to consider if parent compounds linking different positions of the glucose may have different phloem mobilities. In the present work, four novel glucosyl conjugates were designed and synthesized by linking the fipronil to C-2, C-3, C-4, and C-6 of glucose via click chemistry to study the effects of the different positions of glucose on phloem mobility.

MATERIALS AND METHODS

General Information for Synthesis. Melting points were determined on a FP62 digital micro melting point apparatus. Nuclear magnetic resonance (NMR) spectra were obtained on a Bruker AV-600 instrument. Deuterated solvents were obtained from Cambridge Isotope Laboratories (Andover, MA). CD_3OD and $CDCl_3$ solvent peaks (3.31 and 7.26 ppm for ^1H and 49.0 and 77.0 ppm for ^{13}C, respectively) were used as internal chemical shift references. The mass spectra (MS) of new compounds were obtained by a Waters ZQ 4000 with electronspray ionization (ESI) or a Bruker maXis 4G ESI-Q-TOF mass spectrometer. Data were reported as *m*/*z*. Silica gel was used for column chromatography. Reagents and anhydrous solvents were used as purchased without further purification.

Plant Materials. Castor bean seeds (*R. communis*) No. 9, obtained from the Agricultural Science Academy of Zibo, Shandong, China, were sown and grown as previously described.[6] Six-day-old seedlings (the hypocotyl at about 20 mm in length) were relatively selected for further experiments.

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Table 1. Properties of the Tested Compounds[a]

properties	GTF	2-GTF	3-GTF	4-GTF	5-GTF
MW	680.37	680.37	680.37	680.37	680.37
log K_{ow}	2.06	1.11	1.42	1.41	1.58
pK_a	12.8 ± 0.7	11.9 ± 0.7	12.0 ± 0.7	12.0 ± 0.7	12.1 ± 0.7
HBD	5	5	5	5	5
HBA	13	13	13	13	13

[a]The physicochemical properties of tested compounds were predicted by ACD Laboratories Percepta program, version 14.0. Log K_{ow} and pK_a values were classic values. 2-GTF, 3-GTF, 4-GTF, and 6-GTF mean that fipronil was conjugated at the C-2, C-3, C-4, and C-6 positions of glucose, respectively. All values (MW, HBD, and HBA) are the same for the different compounds.

Membrane Potential Measurements. Plasma membrane potential of the protoplasts of *R. communis* cotyledons was performed with flow cytometric analysis using a fluorescent membrane potential indicator dye bis(1,3-dibutylbarbituric acid)-trimethine oxonol [DiBAC4(3)]. The protoplasts were incubated with buffer solution without (control) or with the five glucosyl fipronil conjugates at 100 μM concentration for 5 h, and then the protoplast suspension was co-incubated with 3 μM DiBAC4(3) for 30 min at 28 °C prior to flow cytometric analysis.

Phloem Sap Collection and Analysis. The seedlings with 6 days of development were prepared for phloem sap collection. The phloem sap was collected from the upper part of the hypocotyl, which was similar to that recently described.[6,14] The cotyledons were removed from endosperm carefully without bending or crushing the cotyledons. The cotyledons were incubated in buffered solution supplemented with 100 μM glucosyl fipronil conjugtes. After 1 h of preincubation, the hypocotyl was severed in the hook region for phloem exudation. Phloem sap was collected from the upper part of the hypocotyl at a 1 h interval for 5 h and was stored at 4 °C until analysis.[15]

The collected phloem sap, diluted with pure water [1:4 (v/v) phloem sap/H_2O], was quantified by an Agilent 1100 HPLC system. Separations were made with a C8 reversed-phase column (5 μm, 250 × 4.6 mm inner diameter, Agilent Co.) at 30 °C. The injection was 10 μL, and the flow rate was 1 mL/min. The elution system consisted of acetonitrile and water (50:50, v/v). The conjugates detected in the phloem sap were further identified using ultra-performance liquid chromatography–mass spectrometry (UPLC–MS, Waters, Milford, MA). Samples were separated using an ACQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 100 mm, Waters). The parameters were similar to our previously reported method.[16]

Physicochemical Properties. The physicochemical properties [molecular weight (MW), ionization constant in aqueous solution (pK_a), octanol/water partitioning coefficient (log K_{ow}), number of hydrogen bond donors (HBDs), and number of hydrogen bond acceptors (HBAs)] of different positions of glucose conjugates were predicted using ACD LogD suite version 14.0 software (Table 1).

*Synthesis of 1,3,4,6-Tetra-O-acetyl-2-azido-2-deoxy-β-D-glucoside (**2a**) (Scheme 1).* 2-Azido-2-deoxy-D-glucose (205 mg, 1 mmol) was dissolved in pyridine (5 mL), and then acetic anhydride (Ac_2O, 3 mL) was added with catalytic dimethylaminopyridine (DMAP, 6.1 mg, 0.05 mmol). After the solvents were stirred for 12 h at room temperature, they were removed under reduced pressure and the residue was diluted with CH_2Cl_2. The solution was washed with 1 M HCl and saturated $NaHCO_3$ solution, dried with $MgSO_4$, concentrated under reduced pressure, and purified by column chromatography (1:1 ethyl acetate/hexane) to obtain compound **2a**. White power; yield, 78%; melting point (mp), 80.4 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ, 5.54 (d, J = 8.6 Hz, 1H, H-1), 5.08 (t, J = 9.6 Hz, 1H, H-4), 5.03 (t, J = 9.6 Hz, 1H, H-3), 4.29 (dd, J = 12.5 and 4.5 Hz, 1H, H-6-a), 4.07 (dd, J = 12.5 and 2.1 Hz, 1H, H-6-b), 3.80 (ddd, J = 9.8, 4.5, and 2.2 Hz, 1H, H-5), 3.65 (dd, J = 9.9 and 8.6 Hz, 1H, H-2); ^{13}C NMR (150 MHz, $CDCl_3$) δ, 170.6, 69.8, 169.7, 168.6, 92.6, 72.8, 72.8, 67.9, 62.7, 61.5, 21.0, 20.8, 20.7, 20.6; MS–ESI [M + Na]$^+$, 396.1.

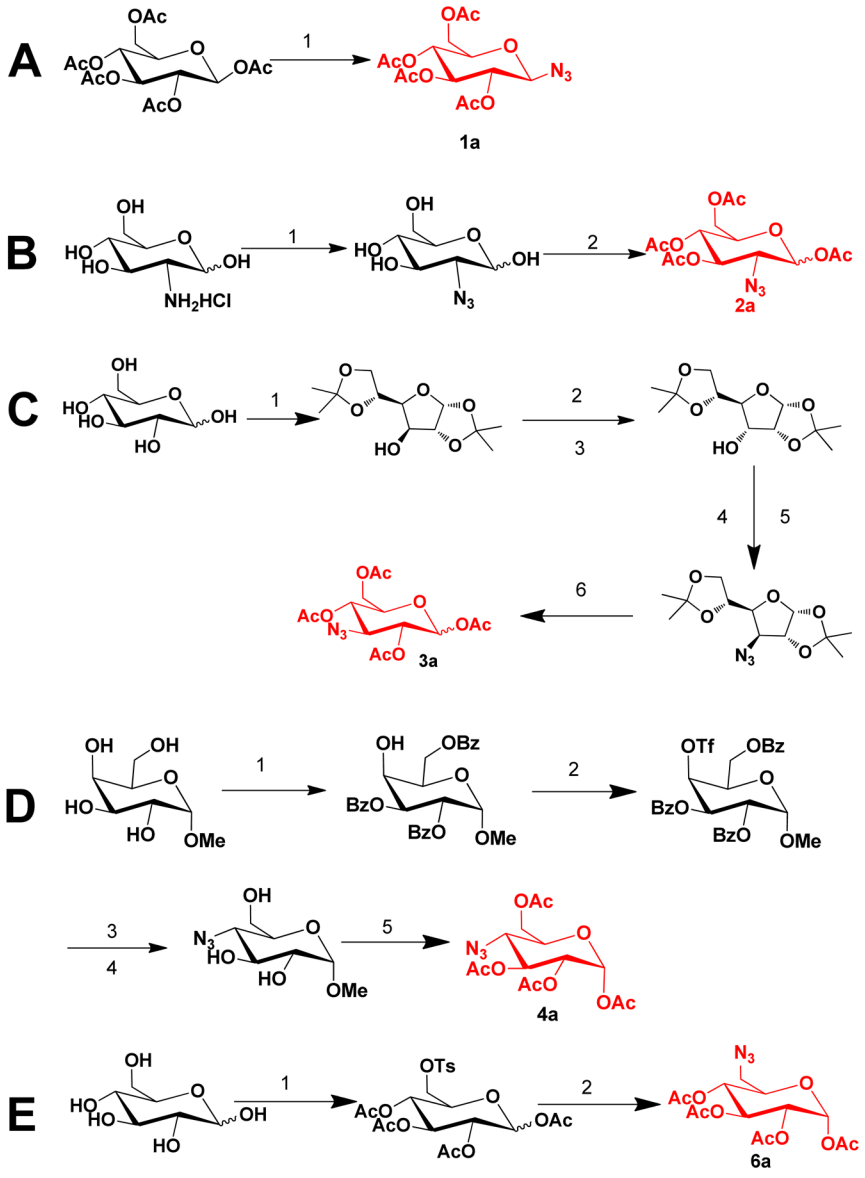
*Synthesis of 1,2,4,6-Tetra-O-acetyl-3-azido-3-deoxy-D-glucoside (Anomers α/β = 1:1) (**3a**).* A solution of 1,2:5,6-di-*O*-isopropylidene-3-azido-3-deoxy-α-D-glucofuranose (2 g, 7 mmol) in 80% CF_3COOH (10 mL) was stirred at room temperature for 3 h. Evaporation of the solvent afforded 3-azido-3-deoxy-D-glucose without further purification. The crude 3-azido-3-deoxy-D-glucose was per-acetylated according to the procedure of compound **2a** to obtain compound **3a**. α anomer: syrup; yield, 35%; ^{1}H NMR ($CDCl_3$, 600 MHz) δ, 6.25 (d, J = 3.6 Hz, 1H, H-1), 4.98 (t, J = 10.1 Hz, 1H, H-4), 4.90 (dd, J = 10.6 and 3.5 Hz, 1H, H-2), 4.17 (dd, J = 12.0 and 4.6 Hz, 1H, H-6-a), 4.05–3.99 (m, 1H, H-6-b), 3.93 (t, J = 10.3 Hz, 1H, H-3), 3.75 (m, H-5), 2.15–2.03 (4 s, 12H, $COCH_3$); ^{13}C NMR ($CDCl_3$, 150 MHz) δ, 171.0, 169.7, 168.6, 87.8, 73.2, 69.9, 69.8, 61.5, 60.5, 21.1, 20.9, 20.8, 20.8. β anomer: syrup; yield, 35%; ^{1}H ($CDCl_3$, 600 MHz) δ, 5.63 (d, J = 8.2 Hz, 1H, H-1), 4.99 (dd, J = 10.1 and 8.2 Hz, 1H, H-2), 4.18 (dd, J = 12.0 and 4.6 Hz, 1H, H-6-a), 4.04–3.96 (m, 3H), 3.66 (t, J = 10.1 Hz, 1H, H-3), 2.15–2.03 (4s, 12H, $COCH_3$); ^{13}C ($CDCl_3$, 150 MHz) δ, 171.0, 169.7, 168.6, 91.3, 69.6, 67.4, 66.0, 64.4, 61.5, 21.1, 21.0, 20.9, 20.8; MS–ESI [M + Na]$^+$, 396.1.

*Synthesis of 1,2,3,6-Tetra-O-acetyl-4-azido-4-deoxy-α-D-glucoside (**4a**).* To a solution of methyl 4-azido-4-deoxy-α-D-glucopyranoside (2 g, 9.13 mmol) in glacial acetic acid (10 mL) and acetic anhydride (10 mL) was added concentrated sulfuric acid (2 mL) dropwise with stirring. The solution was left at room temperature for 48 h. Then, 6.13 g of sodium acetate was added to neutralize the sulfuric acid, and chloroform (100 mL) was added. The mixture was pour into ice–water. The chloroform layer was washed with aqueous $NaHCO_3$ and aqueous NaCl, dried, and evaporated to dryness *in vacuo*. The brown oil was purified by column chromatography (1:1 ethyl acetate/hexane) to obtain compound **4a**. Syrup; yield, 68%; ^{1}H NMR (600 MHz, $CDCl_3$) δ, 6.12 (d, J = 3.6 Hz, 1H, H-1), 5.31 (t, J = 10.0 Hz, 1H, H-3), 4.88 (dd, J = 10.2 and 3.7 Hz, 1H, H-2), 4.18 (dd, J = 12.4 and 1.8 Hz, 1H, H-6-a), 4.12 (dd, J = 12.4 and 3.9 Hz, 1H, H-6-b), 3.80–3.72 (m, 1H, H-5), 3.58 (t, J = 10.2 Hz, 1H, H-4), 2.01 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 1.96 (s, 3H, $COCH_3$), 1.85 (s, 3H, $COCH_3$); ^{13}C (150 MHz, $CDCl_3$) δ, 170.1, 169.5, 169.4, 168.5, 88.8, 70.1, 69.9, 69.0, 62.1, 59.6, 20.5, 20.4, 20.3, 20.1; MS–ESI [M + Na]$^+$, 396.1.

*Synthesis of 1,2,3,4-Tetra-O-acetyl-6-azido-6-deoxy-D-glucopyranose (**6a**).* 1,2,3,4-Tetra-*O*-acetyl-6-*O*-*p*-tolylsulfonyl-D-glucopyranose[17] (7 g, 13.95 mmol) and sodium azide (5.0 g, 154 mmol) dissolved in dry *N*,*N*-dimethylformamide (DMF) (50 mL) were stirred at 50 °C for 4 h and then at room temperature for 16 h. The mixture was poured onto icy water and extracted with ether (4 × 50 mL). The organic extracts were washed twice with water and then dried over calcium chloride. After evaporation to dryness, 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-α-D-glucopyranose (**6a**) was obtained (2.03 g). Needle solid; yield, 39%; mp, 146.2 °C; ^{1}H NMR (600 MHz, $CDCl_3$) δ, 6.33 (d, J = 3.7 Hz, 1H, H-1), 5.44 (t, J = 9.9 Hz, 1H, H-3), 5.09 (t-like, J = 9.6 Hz, 1H, H-4), 5.07 (dd, J = 10.3 and 3.7 Hz, H-2), 4.06 (m, 1H, H-5), 3.38 (dd, J = 13.5 and 2.7 Hz, 1H, H-6-a), 3.28 (dd, J = 13.5 and 5.5 Hz, 1H, H-6-b), 2.17 (s, 3H, $COCH_3$), 2.03 (s, 3H, $COCH_3$), 2.01 (s, 3H, $COCH_3$), 2.00 (s, 3H, $COCH_3$); ^{13}C NMR (150 MHz, $CDCl_3$) δ, 170.3, 169.7, 169.5, 168.8, 88.9, 70.8, 69.7, 69.3, 69.1, 50.8, 20.9, 20.7, 20.7, 20.5; MS–ESI [M + Na]$^+$, 396.1.

General Procedure for Synthesis of the Conjugates 2-GTF, 3-GTF, 4-GTF, and 6-GTF (Scheme 2). Fipronil–alkyne (474 mg, 1 mmol) was added to a vigorously stirred suspension of azide-containing glucoses (373 mg, 1 mmol) in 3 mL of *tert*-butyl alcohol. The reaction was initiated by the addition of a solution of $CuSO_4{\cdot}5H_2O$ (100 mg, 0.4 mmol) and sodium ascorbate (173 mg, 0.8 mmol) in distilled water (3 mL). The deep yellow suspension was stirred vigorously at 50 °C for 3 h. Distilled water (10 mL) was added, and the aqueous layer was

Scheme 1. Synthesis of Azido-Containing Acetylglucoses: (A) 1, $TMSN_3$, $SnCl_4$, CH_2Cl_2; (B) 1, TfN_3, $CuSO_4$, H_2O, MeOH, CH_2Cl_2; 2, Ac_2O, DMAP, Pyridine; (C) 1, Acetone, $ZnCl_2$, H_3PO_4; 2, Pyridinium Dichromate, Ac_2O, CH_2Cl_2; 3, $NaBH_4$, EtOH; 4, Tf_2O, CH_2Cl_2, Pyridine; 5, NaN_3, MeCN; 6, TFA, Ac_2O, DMAP, Pyridine; (D) 1, Benzoyl Chloride, Pyridine; 2, Tf_2O, CH_2Cl_2, Pyridine; 3, NaN_3, MeCN; 4, NaOMe, MeOH; 5, H_2SO_4, Ac_2O; and (E) 1, TsCl, Ac_2O, Pyridine; 2, NaN_3, DMF



extracted with chloroform (10 mL × 3). The combined organic extracts were washed with aqueous sodium hydrogen carbonate and brine, dried with sodium sulfate, filtered, and evaporated *in vacuo*. The crude compound was added to a solution of sodium methoxide in dry methanol (0.05 M, 15 mL). The resultant solution was stirred for 30 min at room temperature. The mixture was neutralized with Amberlite IR 120 (H^+) resin and filtered, and the filtrate was then evaporated. The residues were purified by column chromatography (9:1 CH_2Cl_2/MeOH) to obtain the glucosyl fipronil conjugates.

D-Glucopyranose, 2-Deoxy-2-(4-(((1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((trifluoromethyl)sulfinyl)-3-cyano-1H-pyrazol-5-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)- (2-GTF). White solid; yield, 88%, mp, 151.3 °C; ^{1}H NMR (CD_3OD, 600 MHz) δ, 8.08 (s, 2H), 8.00 (s, 1H), 7.89* (s, 1H), 5.28 (d, J = 3.2 Hz, 1H), 5.11* (d, J = 7.3 Hz, 1H), 4.09−4.21 (m, 2H), 3.93 (d, J = 11.3 Hz, 1H), 3.85 (d, J = 11.5 Hz, 1H), 3.74−3.81 (m, 1H), 3.44−3.55 (m, 2H), 3.32 (brs, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ, 151.7, 144.7, 144.4*, 137.9, 137.9, 137.8*, 136.0, 127.9, 127.9, 125.8, 125.7, 124.4, 123.5, 123.4*, 122.7, 112.2, 97.5, 96.1*, 78.1, 75.4, 73.2, 69.5, 66.7, 62.5, 62.4*, 41.1, 41.0*; HRMS (ESI) calcd for $C_{21}H_{17}Cl_2F_6N_7O_6SNa$ [M + Na]$^+$, 702.0140; found, 702.0135.

D-Glucopyranose, 3-Deoxy-3-(4-(((1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((trifluoromethyl)sulfinyl)-3-cyano-1H-pyrazol-5-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)- (3-GTF). White solid; yield, 80%; mp, 156.8 °C; ^{1}H NMR (CD_3OD, 600 MHz) δ, 8.07 (s, 2H), 7.86 (s, 1H), 7.85* (s, 1H), 5.23 (d, J = 3.1 Hz, 1H), 4.50 (d, J = 3.0 Hz, 2H), 4.47* (d, J = 3.0 Hz, 2H), 4.29 (t, J = 10.1 Hz, 1H), 4.28* (t, J = 10.1 Hz, 1H), 4.04−4.08 (m, 1H), 4.02 (d, J = 10.0 Hz, 1H), 3.96* (d, J = 10.0 Hz, 1H), 3.93−3.96 (m, 1H), 3.89 (d, J = 11.9 Hz, 1H), 3.82* (d, J = 11.9 Hz, 1H), 3.72−3.80 (m, 2H); ^{13}C NMR (CD_3OD, 150 MHz) δ, 151.8, 144.7, 144.4*, 137.9, 136.3*, 136.0, 135.8, 127.9, 127.8, 125.9, 125.7, 124.5, 123.5, 122.5 112.1, 93.4, 79.2, 73.4, 71.7, 69.4, 67.8, 62.3, 41.0; HRMS (ESI) calcd for $C_{21}H_{17}Cl_2F_6N_7O_6SNa$ [M + Na]$^+$, 702.0140; found, 702.0135.

D-Glucopyranose, 4-Deoxy-4-(4-(((1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((trifluoromethyl)sulfinyl)-3-cyano-1H-pyrazol-5-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)- (4-GTF). White solid; yield, 78%, mp, 177.9 °C; ^{1}H NMR (CD_3OD, 600 MHz) δ, 8.08 (s, 2H), 7.88 (d, J = 3.1 Hz, 1H), 7.86* (d, J = 3.1 Hz, 1H), 5.27 (d,

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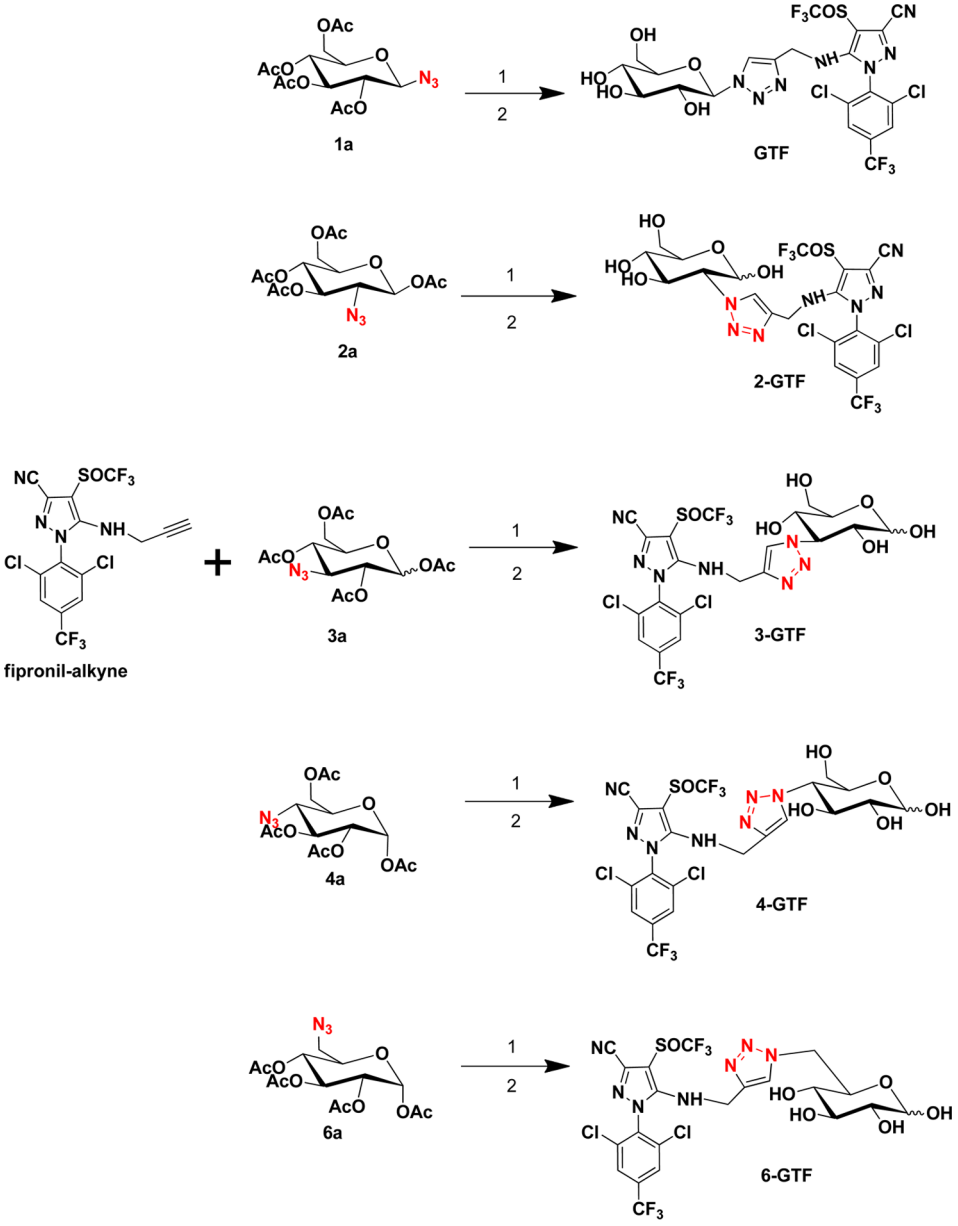
Scheme 2. Synthesis of Fipronil Conjugates for Replacement of C-1, C-2, C-3, C-4, and C-6 of D-Glucose: 1, *tert*-Butyl Alcohol, H_2O, $CuSO_4 \cdot 5H_2O$, Sodium Ascorbate, 50 °C, 3 h; 2, NaOMe, MeOH; Room Temperature, 30 min



J = 3.1 Hz, 1H), 4.47−4.49 (m, 2H), 4.32−4.37* (m, 2H), 4.09 (dd, J = 9.1, 6.0 Hz, 1H), 4.02−4.05* (m, 1H), 3.53 (dd, J = 9.4, 3.6 Hz, 1H), 3.47−3.50 (m, 2H), 3.29 (t, J = 9.1 Hz, 1H), 3.29* (t, J = 9.1 Hz, 1H), 3.11−3.18 (m, 2H); ^{13}C NMR (CD_3OD, 150 MHz) δ, 151.8, 144.6, 137.9, 136.2, 136.0, 127.9, 127.8, 125.5, 125.3, 125.2, 124.5, 122.7, 112.1, 98.3, 94.2*, 76.9, 75.8, 75.3*, 74.4, 72.0, 71.1, 63.8, 63.6*, 61.6, 61.5*, 41.0, 40.9*; HRMS (ESI) calcd for $C_{21}H_{17}Cl_2F_6N_7O_6SNa$ $[M + Na]^+$, 702.0140; found, 702.0135.

D-Glucopyranose, 6-Deoxy-6-(4-(((1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((trifluoromethyl)sulfinyl)-3-cyano-1H-pyrazol-5-yl)amino)methyl)-1H-1,2,3-triazol-1-yl)- (6-GTF). White solid; yield, 88%, mp, 188.4 °C; ^{1}H NMR (CD_3OD, 600 MHz) δ, 8.08 (s, 2H), 7.89 (d, J = 3.1 Hz, 1H), 7.84* (d, J = 3.1 Hz, 1H), 5.06 (d, J = 3.2 Hz, 1H), 4.43−4.46 (m, 2H), 4.06−4.11 (m, 1H), 3.68 (d, J = 9.2 Hz, 1H), 3.68* (d, J = 9.2 Hz, 1H), 3.28−3.39 (m, 2H), 3.10 (t, J = 9.2 Hz, 1H), 3.04* (t, J = 9.2 Hz, 1H); ^{13}C NMR (CD_3OD, 150 MHz) δ, 151.7, 144.9, 137.8, 137.7, 135.9, 127.9, 127.8, 127.8, 125.4, 125.3, 124.4, 122.6, 112.1, 98.2, 94.0*, 77.6, 76.0, 75.6, 74.5, 73.5, 72.7, 72.6, 71.0, 52.3, 52.3*, 41.0, 40.9*. HRMS (ESI) calcd for $C_{21}H_{17}Cl_2F_6N_7O_6SNa$ $[M + Na]^+$, 702.0140; found, 702.0135.

RESULTS AND DISCUSSION

Various strategies for increasing the phloem mobility of pesticides have been considered. A feasible strategy is the use of endogenous transporter proteins to carry pesticides. In this work, D-glucose was chosen as a vector for several reasons. First, it is the most abundant and important monosaccharide in nature. Second, it could be transported by various monosaccharide transporters and their homologues through diverse plant species.[18] Third, D-glucose confers moderate phloem mobility to the non-phloem-mobile insecticide fipronil, as presented in our previous paper.[9,16] In the present work, to evaluate the effects of the different positions of glucose on phloem mobility, four novel GTF conjugates, namely, 2-GTF, 3-GTF, 4-GTF, and 6-GTF, were synthesized via click chemistry based on the synthetic method of GTF.[9] All of the novel structures were confirmed via ^{1}H and ^{13}C NMR spectroscopy and ESI mass spectrometry.

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Synthesis of Azido-Containing Acetylglucoses (Scheme 1). 2,3,4,6-Tetra-*O*-acetyl-1-azido-1-deoxy-D-glucoside (**1a**) was prepared from glucose pentaacetate in the presence of azidotrimethylsilane and $SnCl_4$ (Scheme 1A).[19] Glucosamine hydrochloride was transformed into 2-azido-2-deoxy-D-glucose by diazo transfer from triflyl azide (TfN_3). 2-Azido-2-deoxy-D-glucose was subsequently acetylated using acetic anhydride with catalytic DMAP in pyridine to yield 1,3,4,6-tetra-*O*-acetyl-2-azido-2-deoxy-D-glucose (**2a**) (Scheme 1B).[20] The introduction of nitrogen at C-3 of glucose required a double inversion of the configuration. Thus, the usual oxidation/reduction sequence led to the fomation of diacetone allose, which was then converted to azide via a triflation/azide displacement sequence.[21] Treatment of furanose diacetonide with aqueous trifluoroacetic acid was followed by the complete acetylation of the crude products via treatment with acetic anhydride and pyridine, and pyranose acetates were produced as 1,2,4,6-tetra-*O*-acetyl-3-azido-3-deoxy-D-glucosise (**3a**)[22] (Scheme 1C). Methyl α-D-galactopyranoside (1 equiv) was benzoylated by benzoyl chloride (3.5 equiv) in pyridine to link selectively to methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside because the axial C-4 hydroxyl in the favored conformation has a lower reactivity than C-2, C-3, and C-6.[23] Methyl 2,3,6-tri-*O*-benzoyl-α-D-galactopyranoside was then sulfonated by trifluoromethanesulfonic anhydride. The displacement of the trifluoromethanesulfonate by sodium azide in dimethylformamide gave a good yield of methyl 4-azido-2,3,6-tri-*O*-benzoyl-4-deoxy-α-D-glucopyranoside in syrup form.[24] The debenzoylation of methyl 2,3,6-tri-*O*-benzoyl-4-azido-4-deoxy-α-D-glucopy-ranoside with sodium methoxide resulted in crystalline methyl 4-azido-4-deoxy-α-D-glucopyranoside, which was subsequently peracetylated to obtain a syrupy 1,2,3,6-tetra-*O*-acetyl-4-azido-4-deoxy-α-D-glucose (**4a**). 1,2,3,4-Tetraacetyl-6-*O*-*p*-tolylsulfonyl-D-glucopyranose was prepared from D-glucose as previously described.[17] Conventional azidation in the presence of dimethylformamide resulted in the formation of 1,2,3,4-tetra-*O*-acetyl-6-azido-6-deoxy-D-glucopyranose (**6a**).[25]

Synthesis and Characterization of GTF Conjugates. The synthetic method of GTF conjugates was proposed by Yang et al.[9] To prepare these conjugates via click chemistry, tetraacetylglucosides containing the azide group were synthesized according to the same method mentioned above (Scheme 1). Propargyl was introduced into fipronil according to a previous method by Yang et al.[9] as a coupling partner. In Scheme 2, the $CuSO_4{\cdot}5H_2O$/ascorbate click reaction system was explored to prepare the acetyl glucose-containing fipronil conjugates, which were O-deacetylated via the Zemplén procedure to obtain the target products.

The ^{1}H NMR spectra of these compounds are complicated because fipronil has an asymmetrical sulfoxide and forms two enantiomers.[26,27] In addition, the α,β configuration of the glucosyl group also exists. Their configurations were confirmed on the basis of a chemical shift, coupling constants, and band shape of H-1. For example, the NMR signals of product 2-GTF were immediately measured after the dissolution of the glucosyl conjugates in denatured methanol. A one-proton doublet was observed at 5.28 and $J_{1,2}$ = 3.2 Hz. These results indicate that the substance was in the α-D-pyranose anomer form. The second doublet, observed at 5.11 and $J_{1,2}$ = 7.3 Hz, was assigned to H-1 of the β-D-pyranose anomer. The characteristic peaks at 8.00 in the ^{1}H NMR spectra and the peaks at 127.9 and 123.5 in the ^{13}C NMR spectra appeared after reaction. These results demonstrate that 1,2,3-triazole was produced and the target conjugate was obtained. The analysis via ESI−MS spectroscopy showed a specific peak at m/z 702.0135 $[M + Na]^+$, from which the MW was estimated at 702.0140 $[M + Na]^+$. From these results, the synthesized product was identified as 2-GTF.

Phloem Mobility. The castor bean seedling system is an ideal biological model to evaluate the phloem systemicity of xenobiotics because of its thin and highly permeable cuticle, which facilitates molecule diffusion to and within the cotyledon apoplast system.[28] The system was adopted to test the phloem mobility of GTF, 2-GTF, 3-GTF, 4-GTF, and 6-GTF. As presented in a previous study,[9] the plasma membrane potential of the conjugates was measured via flow cytometric analysis with DiBAC4(3) as the fluorescent dye indicator.[29] In comparison to the control sample, the relative fluorescence data did not have any remarkable changes during the treatment period (Figure 1).

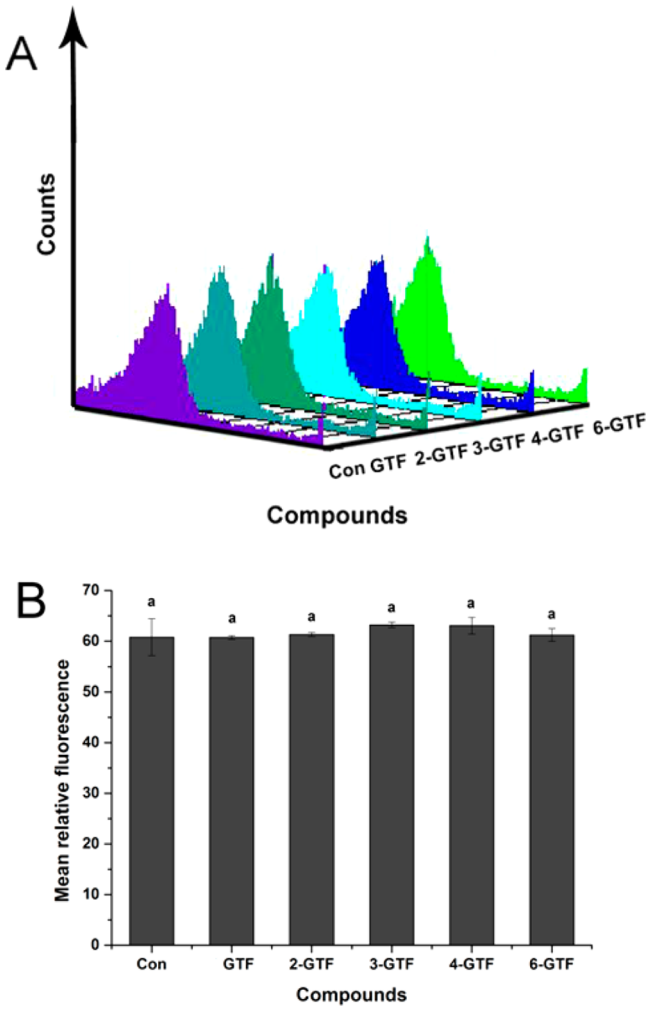

Figure 1. Plasma membrane potential in the protoplasts of *R. communis* cotyledons after 6 h of treatment. (A) Fluorescent intensity histogram marked DiBAC4(3) of the treatment of tested compounds and the control (Con). (B) Relative fluorescence of the treatment of tested compounds and the control (Con). The protoplasts were suspended in a buffered solution at pH 5.5 with or without (control) tested compounds (100 μM) for 5 h. The protoplasts were analyzed by flow cytometry for DiBAC4(3) fluorescence. The data [mean ± standard error (SE); n = 3] within a column are not significantly different, as shown by the Kruskal−Wallis test ($p > 0.05$).

The results indicate that the five conjugates have no depolarizing effects on the transmembrane potential. Therefore, the five

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conjugates were not phytotoxic in the phloem mobility test during the 5 h experimental period.

After a 5 h incubation (including the 1 h pre-incubation period), the conjugates were clearly detected in the phloem sap. The phloem sap determination results indicate that the five conjugates (GTF, 2-GTF, 3-GTF, 4-GTF, and 6-GTF) all exhibited moderate phloem mobility in *Ricinus* seedlings (Figure 2). The GTF conjugates in the phloem sap were further

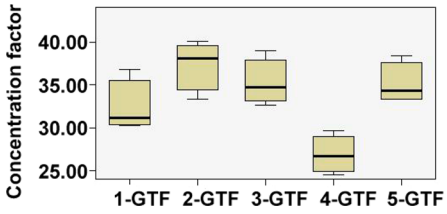

Figure 2. Phloem exudation collection of 4 sets of 12 castor bean seedlings for each conjugate at 5 h (mean ± SE). The Kruskal–Wallis tests at a 5% probability level were used to determine statistical differences among treatments ($p < 0.05$).

identified with UPLC–MS (see the figure in the Supporting Information). The UPLC–MS results indicated that the fipronil parent molecule was not detected in the phloem sap.

The "rule of five", which is widely adopted in the pharmaceutical industry, can also be used to predict the diffusion of endogenous molecules or xenobiotics through plant and animal membranes.[30] According to this rule, the passive absorption of small molecules is more likely to occur when their MW, log*P*, number of hydrogen bond donors, and number of hydrogen bond acceptors are less than 500 Da, 5, 5, and 10, respectively. Given the physical properties of the five conjugates (Table 1), their diffusion through the membranes should be very low. Models based on the physicochemical properties of pesticides are widely used to predict their phloem mobility. The Kleier model is widely used to predict the phloem systemicity of a compound based on the physicochemical properties (pK_a and log K_{ow}) of xenobiotics.[14,16,28] According to Kleier's prediction model, the physical properties of the five conjugates (Table 1) in terms of log K_{ow} and pK_a are found in non-mobile areas (Figure 3). In general, based on the "rule of five" and the Kleier prediction model, the five conjugates should have a low diffusion through the membrane and no phloem mobility should be observed. However, the experimental results of the phloem sap analysis violated the "rule of five" and the Kleier model. Specific carrier processes were probably involved in the phloem transport, as demonstrated in the phloem transport of GTF in *Ricinus* seedlings, which involves an active carrier-mediated mechanism that effectively contributes to GTF phloem loading.[16]

Among these conjugates, the analysis result showed that linking the fipronil to C-2, C-3, C-4, and C-6 of glucose affected phloem mobility of GTF conjugates. The concentrations of GTF, 2-GTF, 3-GTF, 4-GTF, and 6-GTF in phloem sap were 32.35 ± 1.51, 37.37 ± 1.44, 35.25 ± 1.32, 26.87 ± 1.07, and 35.10 ± 1.19 μM, respectively (Figure 2). In a previous work, sugar transporters have been shown to transport many glucose derivatives and glucose conjugates across the membrane. 2-NBDG is incorporated into mammalian cells through glucose transporters in a time-, concentration-, and temperature-dependent manner.[31] The uptake of IPNG into tobacco cells involves an active transport process.[32] Specifically, GTF can be mediated by sugar carrier systems during its phloem loading. The glucose conjugates (2-GTF, 3-GTF, 4-GTF, and 6-GTF) are isomers of GTF and

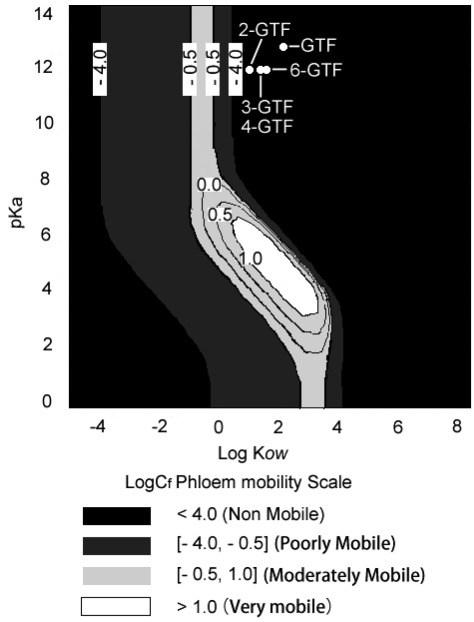

Figure 3. Prediction of phloem mobility of tested compounds (GTF, 2-GTF, 3-GTF, 4-GTF, and 6-GTF) using the Kleier map (log C_f as a function of log K_{ow} and pK_a) according to Kleier;[3,5] plant parameters are for a short plant.[3] Log K_{ow} and pK_a were calculated by the ACD Laboratories Percepta program, version 14.0, and log K_{ow} and pK_a values were classic values.

have very similar properties with each other (Table 1). Therefore, we speculate that sugar carrier systems are involved in the phloem loading of 2-GTF, 3-GTF, 4-GTF, and 6-GTF. Moreover, glucosyl derivatives, which are conjugated at different carbons of glucose, have a significant influence on affinities for sugar carriers. Among the O-methylsulfonyl derivatives of D-glucose, mesylation at C-4 and C-6 of glucose resulted in a slightly diminished affinity for the GLUT1, whereas mesylation at C-3 led to the complete loss of affinity.[33] Therefore, their phloem mobility difference of glucosyl conjugates might be caused by their different affinities for sugar carriers.

In conclusion, four new conjugates containing both fipronil and glucose moieties were synthesized via click chemistry. The phloem mobility tests in the 5 h experimental period demonstrated that the conjugates could enter into the phloem and exhibit moderate phloem mobility without being degraded. Results show that the phloem mobility was closely related to the position of the glucose. Crop protection and production technology have given increasing attention to the effective transport of pesticides through the phloem. Therefore, understanding the influence of the positions of glucose on phloem mobility provides a foundation for synthesis of excellent phloem-mobile sugar pesticides.

■ ASSOCIATED CONTENT

Supporting Information

UPLC–MS indicating that the GTF conjugates were not degraded in the phloem sap during the 5 h experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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