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Abstract

BACKGROUND: Potato (*Solanum tuberosum*) is the fourth culture in the world and is widely used in the agri-food industries. They generate by-products where α-chaconine and α-solanine, the two major solanidine based glycoalkaloids of potato, are present. As secondary metabolites, they play an important role in the protecting system of potato and are involved in plant protection against insects. To add value to these by-products, we described herein new glycoalkaloids that could have phytosanitary properties.

RESULTS: Solanidine, as a renewable source, was modified with an azido linker and coupled by Copper catalyzed alkyne azide cycloaddition (CuAAC) to alkynyl derivatives of the monosaccharides found in the natural potato glycoalkakoids: D-glucose, D-galactose and L-rhamnose. The efficacy of our compounds was evaluated on the potato aphid *Macrosiphum euphorbiae*. The synthetic compounds have stronger aphicidal properties against nymphs than unmodified solanidine. They also showed strong aphicidal activities on adults and a negative impact on fecundity.

CONCLUSION: Our synthetic neoglycoalkaloids affected *Macrosiphum euphorbiae* survival at the nymphal stage as well as at the adult stage. Furthermore, they induced a decrease of fecundity. Our results show that chemical modifications of by-products may afford new sustainable compounds for crop and plant protection.

Keywords

glycoalkaloid, insecticide, solanidine, synthesis, solanum, potato.

Running Title: Solanidine Derivatives

1 Introduction

Plants constitute an important source of bioactive compounds, and one of the best alternatives to fossil resources in the development of sustainable chemistry. Potato is the fourth world agricultural production with about 380 million metric tons (FAOSTAT, http://www.fao.org/faostat/en/#data/QC). The consumption per capita increases each year and goes along with the consumption of processed products, to the detriment of fresh potato. In the last decades, the development of industrial potato-based food products led to a large amount of by-products (mainly skin and tuber). Their management is an environmental and economic challenge. Nowadays, these by-products are partially used as farm animal feed, in the production of fuel-grade ethanol and in anaerobic digesters. However, they may be a source of bioactive compounds such as phenolic compounds and glycoalkaloids[1] which may be considered as high added-value residues for industrial purposes.

Glycoalkaloids are secondary metabolites found in solanaceae species. α-Chaconine and α-solanine are the main glycoalkaloids found in potato. They have the same aglycone, solanidine, but they differ in their saccharide moiety, chacotriose and solatriose respectively (Figure S1). The glycoalkaloids content is highly dependent on the varieties of potatoes. They are located all over the plant: tubers, sprouts or aerial parts as reported by Friedman[2] and analyzed by mass spectrometry.[3] Their amounts vary from a few milligrams per kilogram of fresh matter in the inner part of the tuber to several grams per kilogram of fresh matter in the sprouts.

As secondary metabolites, α-chaconine and α-solanine play an important role in the immune system of potato and are involved in plant protection against insects.[4] The potato leafhopper (*Empoasca fabae*) is sensitive to glycoalkaloids. α-Chaconine and α-solanine at 0.09% (about

1 mM) have a toxic effect with a mortality of 59% and 8%, respectively. When the concentration is increased to 0.27%, mortality was higher than 80% for both compounds.[5] A toxic effect was also observed against beetle (*Trogoderma granarium*). This pest of stored grains and cereal products is drastically affected after 96 h, by a topical application of α-chaconine (LD_{50} = 18.1 µg/mg insect) and α-solanine (LD_{50} = 22.5 µg/mg insect) as shown by Nenaah.[6] α-Chaconine or α-solanine containing phyto extracts also affect the development and reproduction of *Drosophila melanogaster*.[7] On the contrary, colorado potato beetle (*Leptinotarsa decemlineata*) survival is not affected by the two glycoalkaloids.[8]

Aphids can be harmful to a lot of cultures because of their phytophagous activity, but also as carriers of pathogens leading to plant diseases. The survival of *Schizaphis graminum*, an aphid enfeoffed to *Poaceae* plants, is affected by either α-chaconine or α-solanine by ingestion. After 24 h on an artificial diet, 48% of aphids died with α-chaconine at 250 µM and 61% with α-solanine at 250 µM.[9] Similarly, an extract of potato glycoalkaloids at 160 $mg.L^{-1}$ increased mortality of the green peach aphid, *Myzus persicae*.[10] The authors also demonstrated that glycoalkaloids alter life history traits of this aphid by reducing diet uptake and fecundity. These results have been partially confirmed for another aphid species, the potato aphid *Macrosiphum euphorbiae*.[11] α-Chaconine at 250 ppm allowed the reproduction to decrease while α-solanine did not. None of these two glycoalkaloids induced mortality, even at high concentration. Nevertheless, the authors demonstrated a toxic effect of solanidine (the aglycone of α-chaconine and α-solanine) at 250 ppm.

The toxicity of potato glycoalkaloids is mainly due to three mechanisms of action. They are cytotoxic (affecting the cell membranes containing sterols) with a synergistic action,[12] they disturb the ionic flux,[13] and they inhibit cholinesterases.[14]

Considering the literature, it is possible to evaluate more precisely the influence of the saccharide moiety. Inhibition of cholinesterases by solanidine glycoalkaloids is slightly dependent on the saccharide moiety. α-Chaconine and α-solanine inhibited acetyl or butyrylcholinesterase to the same level, few more than β_2-chaconine.[15] The cytotoxicity of solanidine glycoalkaloids is more dependent on the saccharide moiety, as it has been shown on liposome models.[16] α-Chaconine showed significant lytic activity on liposomes while α-solanine or β_2-chaconine did not. As a consequence, the toxicity of the solanidine based glycoalkaloids can be affected by the sugar composition. α-Chaconine and β_1-chaconine were more toxic on frog embryos than α-solanine, β_2-chaconine or γ-chaconine.[17] The same trend was observed for teratogenicity. α-Chaconine and β_1-chaconine were more teratogenic for frog embryos than β_2-chaconine or γ-chaconine.

Synthetically modified solanidine derivatives could be the base of a new sustainable crop and plant protection strategy. Herein, we describe the synthesis of new glycoalkaloids starting from solanidine as a renewable source of hemisynthetic bioactive molecules. We combined solanidine and the monosaccharides found in the natural potato glycoalkakoids (chacotriose and solatriose): D-glucose, D-galactose and L-rhamnose. A spacer arm was incorporated between the aglycon and the saccharide moiety. The efficiency of our compounds was evaluated on the aphid *Macrosiphum euphorbiae*. As this insect is polyphagous, found worldwide and a main pest of many cultivated plants such as potato, tomato, peas, etc., it is a good model for testing new sustainable pesticides. The insecticidal activity of our compounds was compared to that of the solanidine in order to evaluate the effects of structural modifications.

2 Materials and Methods

2.1 Chemicals and Instruments

Solanidine was extracted from potato as described previously.[18] Skin and sprouts from *S. tuberosum* cv. Pompadour was supplied by the Comité Nord Plants de Pomme de Terre and was used as starting material. All purchased materials were used without further purification. Dichloromethane was distilled from calcium hydride and tetrahydrofuran over sodium and benzophenone. Analytical thin-layer chromatography (TLC) was carried out using Merck D.C.-Alufolien Kieselgel 60 F_{254}. Flash chromatography (FC) was performed on a Reveleris iES System supplied by Grace (USA) using pre-packed silica cartridges and ELSD/UV detection.

2.2 Synthetic procedures

2.2.1 Procedure A - 1,3-dipolar cycloaddition (Meldal conditions). The azido solanidine derivative (1 mmol) and the peracetylated propargyl sugar derivative (1.2 mmol) were dissolved in toluene (25 mL). N,N-Diisopropylethylamine (DIPEA) (1.2 mmol) and CuI (0.2 mmol) were added and the reaction mixture was stirred for 8 h at 110 °C. After filtration through celite, the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (1:0 to 1:1 cyclohexane-EtOAc containing 0.1% Et_3N). The purified product was finally dried in a desiccator overnight.

2.2.2 Procedure B – Deacetylation with sodium methanolate. Sodium (1 mmol) was added to MeOH (45 mL). The sodium methanolate solution obtained was then added to a solution of the acetylated compound (1 mmol) in MeOH (45 mL). The reaction mixture was stirred at room temperature for 4 h. Acid resin Amberlite® IR120 [H^+] was added until a pH value of 5-6 was reached. The reaction mixture was stirred at room temperature for 30 min and then filtered to remove the resin. The solvent was removed under reduced pressure.

2.2.3 Propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (2). 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose (**1**, 25.62 mmol, 10.0 g) was dissolved in anhydrous CH_2Cl_2 (200 mL) under argon. Propargyl alcohol (30.72 mmol, 1.82 mL) was added followed by $BF_3.Et_2O$ (102.48 mmol, 12.64 mL). The mixture was stirred for 2 h at room temperature. Then, potassium carbonate (38.43 mmol, 5.31 g) was added and the reaction stirred for 30 min at room temperature. After filtration, the mixture was washed with distilled water (2 x 200 mL). The aqueous layers were combined and extracted with CH_2Cl_2 (2 x 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was dissolved in a minimum volume of CH_2Cl_2, then cyclohexane was added until the precipitation started. The mixture was stirred for 20 min at room temperature. Compound **2** was obtained after filtration through sintered glass, with 84% yield (8.3 g) as a white solid.

2.2.4 Propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (4). 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose (**3**, 12.81 mmol, 5.0 g) and silver trifluoroacetate (19.21 mmol, 4.28 g) were dissolved in anhydrous CH_2Cl_2 (150 mL). Propargyl alcohol was added (19.21 mmol, 1.13 mL) followed by $SnCl_4$ (38.43 mmol, 4.5 mL). The reaction mixture was stirred for 1.5 h at room temperature. CH_2Cl_2 (400 mL) was added and the mixture was washed with, successively, saturated solution of $NaHCO_3$ (300 mL), distilled water (3 x 300 mL) and brine (300 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (1:0 to 1:1 cyclohexane-EtOAc). Compound **4** was obtained in 87% yield (4.3 g) as a white solid.

2.2.5 1,2,3,4-tetra-*O*-acetyl-L-rhamnopyranose (6). L-rhamnose (**5**, 109.0 mmol, 20.0 g) was dissolved in pyridine (80 mL). Acetic anhydride (80 mL) was then added and the reaction mixture was stirred for 15 h at room temperature. After evaporation of the solvent, the residue was dissolved in EtOAc (250 mL) and washed with distilled water (3 x 100 mL). The organic layer was dried over $MgSO_4$, filtered, and the solvent was removed under reduced pressure. Compound **6** was obtained in 98% yield (35.1 g) as a colorless syrup (α/β: 86/14).

2.2.6 Propargyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside (7). Compound **6** (11.14 mmol, 3.8 g) was dissolved in anhydrous CH_2Cl_2 (50 mL) under argon. Propargylic alcohol (13.72 mmol, 0.81 mL) was added followed by $BF_3.Et_2O$ (45.74 mmol, 5.65 mL). The mixture was stirred for 2 h at room temperature. Then, potassium carbonate (17.15 mmol, 2.37 g) was added and the reaction stirred for 30 min at room temperature. After filtration, the solution was diluted with CH_2Cl_2 (100 mL) and washed with distilled water (2 x 100 mL). The aqueous layers were combined and extracted with CH_2Cl_2 (2 x 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (1:0 to 3:2 cyclohexane-EtOAc). Compound **7** was obtained in 79% yield (2.9 g) as a white solid.

2.2.7 3-azidopropan-1-ol (9). 3-chloropropan-1-ol (**8**, 0.11 mol, 10.0 g) was dissolved in distilled water (45 mL). Sodium azide (0.21 mmol, 13.78 g) was added and the reaction mixture was stirred for 15 h at 80°C. After cooling to room temperature, the mixture was extracted with diethyl ether (3 x 50 mL). The organic layers were combined and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure. Compound **9** was obtained in 99% yield (11.0 g) as a colorless liquid.

2.2.8 3-azidopropyl *p*-toluenesulfonate (10). Compound **9** (0.11 mol, 11.5 g) was dissolved in CH_2Cl_2 (110 mL). 4-Dimethylaminopyridine (DMAP) (23.0 mmol, 2.78 g) and Et_3N (0.17 mol, 23.8 mL) were added and the reaction mixture was placed at 0°C. A solution of *p*-Toluenesulfonyl chloride (0.17 mol, 32.41 g), in CH_2Cl_2 (60 mL) was added and the mixture was stirred at room temperature for 15 h. Then, the mixture was diluted with CH_2Cl_2 (200 mL) and washed with, successively, a saturated solution of $NaHCO_3$ (200 mL), a 10% solution of HCl (200 mL) and distilled water (200 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (1:0 to 3:2 cyclohexane-Et_2O). Compound **10** was obtained in 76% yield (21.4 g) as a colorless liquid.

2.2.9 3-*O*-(3-azidopropyl)solanidine (12). Solanidine (**11**, 0.25 mmol, 100 mg) was dissolved in anhydrous THF (4 mL) and the solution was placed under argon. NaH 95% (1.26 mmol, 33 mg) was added and the mixture stirred for 30 min at room temperature. Compound **10** (1.26 mmol, 0.32 g) was then added and the reaction mixture stirred for 48 h at 60 °C. After cooling to room temperature, the solvent was removed under reduced pressure. The residue was dissolved in chloroform and filtered through celite. After concentration under reduced pressure, the crude product was purified by flash chromatography (1:0 to 9:1 $CHCl_3$-MeOH). The product was finally recrystallized in acetonitrile. Compound **12** was obtained in 71% yield (85 mg) as a white solid.

2.2.10 3-*O*-{3-[4-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine (13). Compound **13** was prepared from compound **12** (0.34 mmol, 165 mg) and compound **2** (0.72 mmol, 170 mg) according to the procedure A and obtained in 73% yield (217 mg) as a white solid.

2.2.11 3-*O*-{3-[4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine (14). Compound **14** was prepared from compound **12** (0.21 mmol, 100 mg) and compound **4** (0.25 mmol, 96 mg) according to the procedure A, and obtained in 72% yield (129 mg) as a white solid.

2.2.12 3-*O*-{3-[4-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine (15). Compound **15** was obtained from compound **12** (0.66 mmol, 320 mg) and compound **7** (0.80 mmol, 262 mg) according to the procedure A and obtained in 71% yield (379 mg) as a white solid.

2.2.13 3-*O*-{3-[4-(β-D-glucopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine (16). Compound **16** was prepared from compound **13** (0.14 mmol, 125 mg) according to the procedure C and purified by flash chromatography (1:0 to 4:1 $CHCl_3$-MeOH containing 0.1% Et_3N). Compound **16** was obtained in 68% yield (68 mg) as a white solid.

2.2.14 3-*O*-{3-[4-(β-D-galactopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine (17). Compound **17** was synthesized from compound **14** (0.15 mmol, 129 mg) according to the procedure C purified by flash chromatography (1:0 to 4:1 $CHCl_3$-MeOH containing 0.1% Et_3N). Compound **17** was obtained in 72% yield (76 mg) as a white solid.

2.2.15 3-*O*-{3-[4-(α-L-rhamnopyranosyloxymethyl)-1,2,3-triazol-1-yl]propyl}solanidine (18). Compound **18** was prepared from compound **15** (0.53 mmol, 430 mg) according to the procedure C and purified by flash chromatography (1:0 to 9:2 $CHCl_3$-MeOH containing 0.1% Et_3N). Compound **18** was obtained in 77% yield (279 mg) as a white solid.

2.3 Insects and feeding assays

Macrosiphum euphorbiae was mass-reared on potato plants (*Solanum tuberosum* cv Désirée) in environmental chambers maintained at 20 ± 1° C, 60 ± 5% relative humidity, and a photoperiod of 16:8 h (L:D) to induce parthenogenesis. The colony was initiated from a single virginiparous female supplied by INRA/INSA Villeurbanne (France) in 2004 and initially collected in 1995 from an eggplant field in the Rhône Alpes region (southern France). A standard artificial diet, diet pouches and feeding chambers were prepared as described on the literature.[19,20] It used as a carrier for natural glycoalkaloids (α-chaconine, α-solanine, solanidine) and synthesis derivatives (**16**, **17**, **18**) dilution and as a control. The concentrations of the above compounds incorporated into the artificial diet were 0 (control), 2, 20 and 200 µM. For nymphal survival, pools of synchronized first instar nymphs (less than 24 h old) were obtained from parthenogenetic females placed on pouches of control diet. Groups of five first instar nymphs were then transferred on pouches of each diet with a sample of 50 nymphs per conditions. Survival was recorded every 2 days for 10 days (until nymphs become adults). For adult experiments, the development of synchronized first instar nymphs until adult state was done in control diet for 10 days. Then, groups of five young adults were transferred on pouches of each diet with a sample of 40 nymphs per conditions. Survival and fecundity was recorded every 2 days for 16 days. Pouches of artificial diet were changed every 2 days to avoid bacterial or fungal contamination.[21,22]

2.4 Statistical analyses

Mean values are given with their standard error of the mean (SEM). The effect of compounds and different concentrations on aphid survival (after 10 for nymphs and 16 days for adults) that were not normally distributed was analyzed using a Kruskal–Wallis one-way analysis of

variance (*H*), followed by multiple comparison tests using the R package "nparcomp" (type: Dunnet for comparison to control). Aphid survival was analyzed using a Cox- proportional hazards model. The effect of compounds and different concentrations on aphid fecundity that was not normally distributed was analyzed using a Kruskal–Wallis one-way analysis of variance (*H*), followed by multiple comparison tests using the R package "PMCMR" (pairwise comparison Dunn test). All statistical analyses were carried out using the statistical program "R" (R 3.2.2—R Development Core Team, 2015).

3 Results and Discussion

3.1 Synthetic route of new glycoalkaloids

The hemisynthesis of our new glycoalkaloids was designed with a convergent strategy. Solanidine (isolated by extraction of potato sprouts) was first functionalized with an azido-propyl chain by S_N2 substitution on the 3-OH position. Nucleophilic substitution has been previously used to prepare 3-*O*-steroid derivatives.[23,24] Among the synthetic options, a leaving group have been placed on the linker, the 3-OH acting as the nucleophile, was chosen in order to prevent inversion/epimerization of the chiral carbon 3 atom.

Then, the azido-solanidine derivative was linked to a propargyl glycoside by copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) affording a triazole type spacer arm. CuAAC has several advantages such as regioselectivity and the lack of secondary reactions. The 1,2,3-triazole is stable in biological conditions and, as far as we know, is biocompatible. The use of a triazole as a spacer between an aglycon (diosgenin) and chacotriose has been already described to study the effect on the cytotoxic activity.[25] This modification was conducted by click reaction of an 1-azidochacotriosyl with a propargyl-diosgenin or by the reaction of propargyl chacotrioside with an azido/azidoalkyl diosgenin derivative.

The synthesis had to be simple and efficient. Consequently we chose, as saccharide moiety, the three different monosaccharide units present in the natural potato glycoalkaloids (β-D-glucose, β-D-galactose and α-L-rhamnose). By addition of only one monosaccharide, we could evaluate their individual impact on the activity.

For cytotoxicity activity, the mechanism of lytic action is based on the interaction of steroids alkaloids with sterol containing in membranes.[26] This interaction is followed by the formation of intermolecular hydrogen bonds between saccharide moieties which induces the destruction of membranes. In our strategy, we wanted to balance the lack of some saccharide units by adding a flexible spacer arm which could enhance intermolecular hydrogen bond formation. The presence of the triazole ring, resulting from the click reaction, could result in additional H-bond or CH/π interactions, and the flexible propyl spacer arm could facilitate the interactions between solanidine and the binding site on cholinesterases.

3.2 Synthesis

First, alkynyl glycosides were synthesized. The reaction of 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranose **1** with propargyl alcohol, in the presence of $BF_3.Et_2O$ at room temperature, following a reported procedure,[27] afforded the glycoside **2** (84% yield). For the galactoside derivative **4**, only Praly's conditions[28] with $SnCl_4$ and CF_3CO_2Ag allowed to obtain β-anomer exclusively, in 87% yield. The propargyl α-L-rhamnoside **7** was obtained in 71% overall yield via the peracetate **6** in the conditions used for the glucose derivative.

On the other hand, to obtain the azido-solanidine derivative by nucleophilic substitution, we had to synthesize an appropriate linker with an azido group and a tosylate as leaving group. 3-chloropropanol was azidated with NaN_3 in water, and the resulting 3-azidopropanol was tosylated[29] with 75% overall yield for **10** from **8** (Figure 1).

Then, we could put the different moieties together. The first step was the reaction of solanidine **11** (extract from potato sprout) with 5 equivalents of **10,** afforded the azido-derived solanidine **12** in 71% yield (Figure 1). We were able to purify it using 1:0 to 9:1 $CHCl_3$-CH_3OH containing 0.1% of Et_3N, followed by precipitation in acetonitrile. Acetyl protected propargyl glycosides **2**, **4** and **7** were reacted with solanidine derivative **12** by CuAAC in the presence of CuI and DIPEA, to give the protected coupled derivatives **13** (73%), **14** (72%) and **15** (71%), respectively (Figure 2). Deacetylation of compounds **13**, **14** and **15** afforded the final adducts **16** (68%), **17** (72%) and **18** (77%), respectively.[30]

3.3 Effect of α-solanine, α-chaconine and solanidine on nymphal survival

The effect of the natural compounds α-solanine, α-chaconine and solanidine on *Macrosiphum euphorbiae* was studied using a standard artificial diet as a carrier containing the compound at three different concentrations: 2, 20 and 200 µM. At 10 days, nymphal survival was significantly affected by treatments (Kruskal-Wallis test, $H = 41.751$, df = 9, $P < 0.001$) (Figure 3a). Compared to the control, nymphal survival was significantly reduced at the 200 µM threshold for α-chaconine (54% survival), α-solanine (64%) and solanidine (52%) (multiple nonparametric comparisons, type: Dunnet; $P < 0.001$, $P < 0.01$ and $P < 0.001$, respectively). At 20 µM, the reduction was significant only for solanidine with 64% of survival ($P < 0.01$).

When comparing the different treatments during all the experiment length (supporting information, figure S2), nymphal survival was significantly affected by the added compound (ANOVA Cox model, $\chi^2 = 6.923$, df = 2, $P < 0.05$) and by the concentration (ANOVA Cox model, $\chi^2 = 27.955$, df = 2, $P < 0.001$) indicating a dose dependent effect.

Solanidine at a concentration of 200 µM has been thus used as a positive control in the evaluation of synthetic compounds.

3.4 Effect of synthetic compounds 16, 17 and 18 on nymphal survival.

Synthetic derivatives **16**-**18** were also evaluated at three concentrations: 2, 20 and 200 µM in comparison of solanidine at 200 µM selected from the above experiment. After 10 days, nymphal survival was significantly affected by treatments (Kruskal-Wallis test, $H = 125.55$, df = 9, $P < 0.001$) (Figure 3b). Compared to solanidine at 200 µM (nymphal survival 52%), the glucose-containing molecule **16** displayed higher aphicidal properties at 20 µM and at 200 µM, with 25% of nymphal survival for both concentration (multiple nonparametric comparisons, type: Dunnet; $P < 0.05$). The galactosyl derivative **17** at 2 and 20 µM showed similar effects than solanidine at 200 µM (64% and 34% survival respectively). However, at 200 µM, a significantly higher aphicidal effect (24% survival) was observed (multiple nonparametric comparisons, type: Dunnet; $P < 0.05$). Finally, compared to solanidine at 200 µM, compound **18** did not show any significant difference at 20 µM but showed stronger aphicidal activity at 200 µM with 6% of nymphal survival (multiple nonparametric comparisons, type: Dunnet; $P < 0.001$).

In conclusion, at 20 µM only the glucose-containing molecule **16** exhibited higher activity than solanidine at 200 µM. At equal concentration (200 µM), the three synthetic molecules were significantly more active than solanidine. The rhamnose-containing molecule **18** at 200 µM shows the most significant activity on nymphal survival.

When comparing the different treatments during all the experiment length (supporting information, figure S3), nymphal survival was significantly affected by the compound (ANOVA Cox model, χ^2 = 24.579, df = 3, P < 0.001) and by the concentration (ANOVA Cox model, χ^2 = 132.978, df = 2, P < 0.001) indicating a dose dependent effect. As observed for α-chaconine, α-solanine and solanidine, a severe reduction in nymphal survival was observed within the first 4 days of treatment.

3.5 Effect of synthetic compounds 16, 17 and 18 on adult survival.

Synthetic derivatives **16**-**18** (at 2, 20 and 200 µM) were evaluated on *Macrosiphum euphorbiae* adults (Figure 4a). Compared to the control, adult survival was significantly affected by treatments (Kruskal-Wallis test, H = 130.95, df = 9, P < 0.001) after 16 days. The glucose-containing molecule **16** and the galactosyl derivative **17** exhibited significant aphicidal activity at the 200 µM threshold, 46% and 54% adult survival respectively (multiple nonparametric comparisons, type: Dunnet; P< 0.001 for both compounds). The rhamnosyl derivative **18** showed strong aphicidal properties at 20 µM (ca. 67% survival, multiple nonparametric comparisons, type: Dunnet; P< 0.05) and at 200 µM (ca. 7% survival, multiple nonparametric comparisons, type: Dunnet; P< 0.001).

When comparing the different synthetic glycoalkaloids treatments (Figure 4b), adult survival was significantly affected by the added compound (ANOVA Cox model, χ^2 = 12.367, df = 2, P< 0.01) and by the concentration (ANOVA Cox model, χ^2 = 101.299, df = 2, P< 0.001) indicating a dose dependent effect. Globally, adult survival was significantly lower for the strongest concentrations. The rhamnose-based compound **18** at 200 µM showed a significant decreased of aphid survival compared to all the other treatments (pairwise comparisons using least-squares means, $P < 0.05$).

3.6 Effect of synthetic compounds 16, 17 and 18 on reproduction.

The presence of synthetic glycoalkaloids in the artificial diet affected reproduction (Kruskal-Wallis test, H = 130.95, df = 9, $P < 0.001$) (Figure 5). Overall, reproduction was more affected by the three synthetic compounds at the highest concentrations. At low concentration (2 µM), only the glucosyl derivative **16** decreased significantly the fecundity (pairwise comparison Dunn test, $P < 0.05$), whereas compounds **17** and **18** had no effect ($P > 0.05$). At 20 µM, all three compounds decreased significantly the fecundity to a similar extent, compared to the control. At 200 µM, the three molecules **16**, **17** and **18** had a strong effect. The rhamnosyl derivative **18** showed the strongest activity, significantly higher than **16** and **17** ($P < 0.05$), and with a dose-dependent response.

4 Conclusion

All these results show that our approach to obtain strong aphicidal activity with modified glycoalkaloids is promising. Starting from solanidine, a simple and efficient synthetic strategy allowed to click different sugars in a few steps. The analogs have been designed to include a triazole-containing spacer between the sugar and the alkaloid moieties. This modification

increases the flexibility of the molecule and might facilitate interactions with biological targets. The aphicidal activity of our synthetic neoglycoalkaloids containing only one monosaccharide unit was proven. They affected *Macrosiphum euphorbiae* survival at the nymphal stage as well as at the adult stage. Furthermore, they induced a decrease of fecundity. In addition, our results show the influence of the monosaccharide structure on the activity, as the rhamnosyl derivative **18** is clearly more active than the glucosyl and the galactosyl conjugates **16** and **17** on adult survival and on reproduction. Further studies could shed light on the specific mechanism of these new glycoalkaloid derivatives and to study their effects on other pests. In perspective, it could also be interesting to investigate the feeding behavior of aphids in the presence of our compounds using the electrical penetration graph (EPG) technique.[31] Anyway, our results show that the effect of natural glycoalkaloids can be amplified through structural modifications performed by chemical synthesis, leading to new sustainable compounds for crop and plant protection.

5 Acknowledgments

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6 Supporting Information

Characterization of compounds **2** to **18**. ^{1}H and ^{13}C NMR spectra of compounds **12-18**. Names and chemical structures of natural hydrolysis products of α-chaconine and α-solanine (Figure S1). Cox graphic representations of the nymph survival of *Macrosiphum euphorbiae* reared on diets (Figures S2 and S3). Tables corresponding to graphics (Tables S1, S2, S3).

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Figure captions

Fig. 1. Reagents: (a) NaN_3, H_2O, 80 °C, 15 h, 99%; (b) TsCl, Et_3N, DMAP, CH_2Cl_2, 15 h, 0 °C → rt, 76%; (c) NaH, THF, 60 °C, 24 h, 71%.

Fig. 2. Reagents: (a), **12**, CuI, DIPEA, toluene, 8 h, 110 °C; (b) NaMeO, MeOH, 4 h, rt.

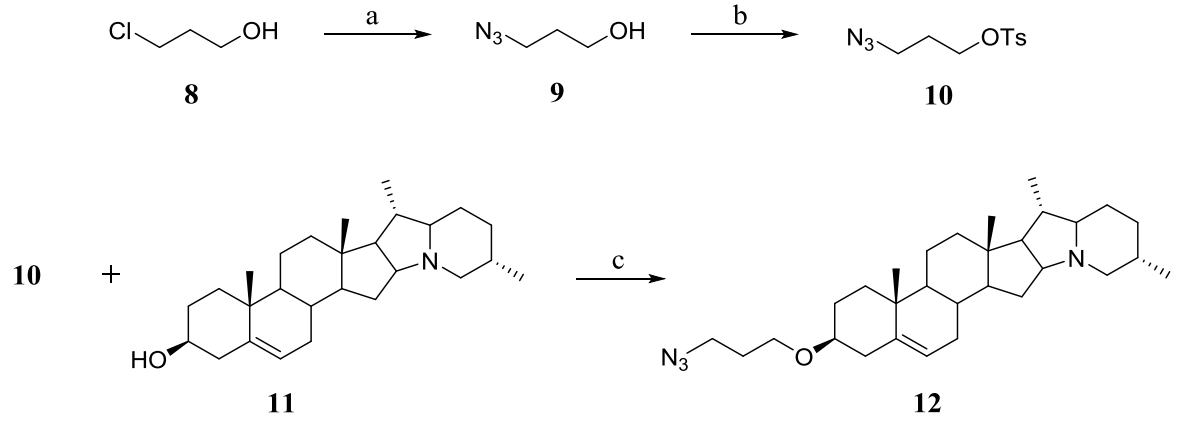
Fig. 3. (a) Nymphal survival of *Macrosiphum euphorbiae* reared on diets containing 2, 20 or 200 μM of α-chaconine (light gray), α-solanine (gray) and solanidine (black) after 10 days of treatment. (b) Nymphal survival of *Macrosiphum euphorbiae* reared on diets containing 2, 20 or 200 μM of **16** (light gray), **17** (gray) and **18** (black) after 10 days of treatment. Asterisks

indicate statistically significant differences between the control (solanidine at 200 μM) and the treatment (multiple nonparametric comparisons, type: Dunnet; *P < 0.05; ** P < 0.01; *** P < 0.001).

Fig. 4. (a) Adult survival of *Macrosiphum euphorbiae* reared on diets containing 2, 20 or 200 μM of compounds **16** (light gray), **17** (gray) and **18** (black) after 16 days of treatment. Asterisks indicate statistically significant differences between the control and the treatment (multiple nonparametric comparisons, type: Dunnet; *P < 0.05; ** P < 0.01; *** P < 0.001). (b) Adult survival of *Macrosiphum euphorbiae* reared on diets containing 2 μM (full line), 20 μM (dashed line) or 200 μM (dotted line) of compounds **16** (light gray), **17** (gray) and **18** (black).

Fig. 5. Total fecundity (± SEM) of *Macrosiphum euphorbiae* reared on diets containing 2 μM, 20 μM or 200 μM of compounds **16** (light gray), **17** (gray) and **18** (black) and control (white). Letters indicate significant differences between treatments associated with Dunn test.

Figure graphics



530 Fig. 1.

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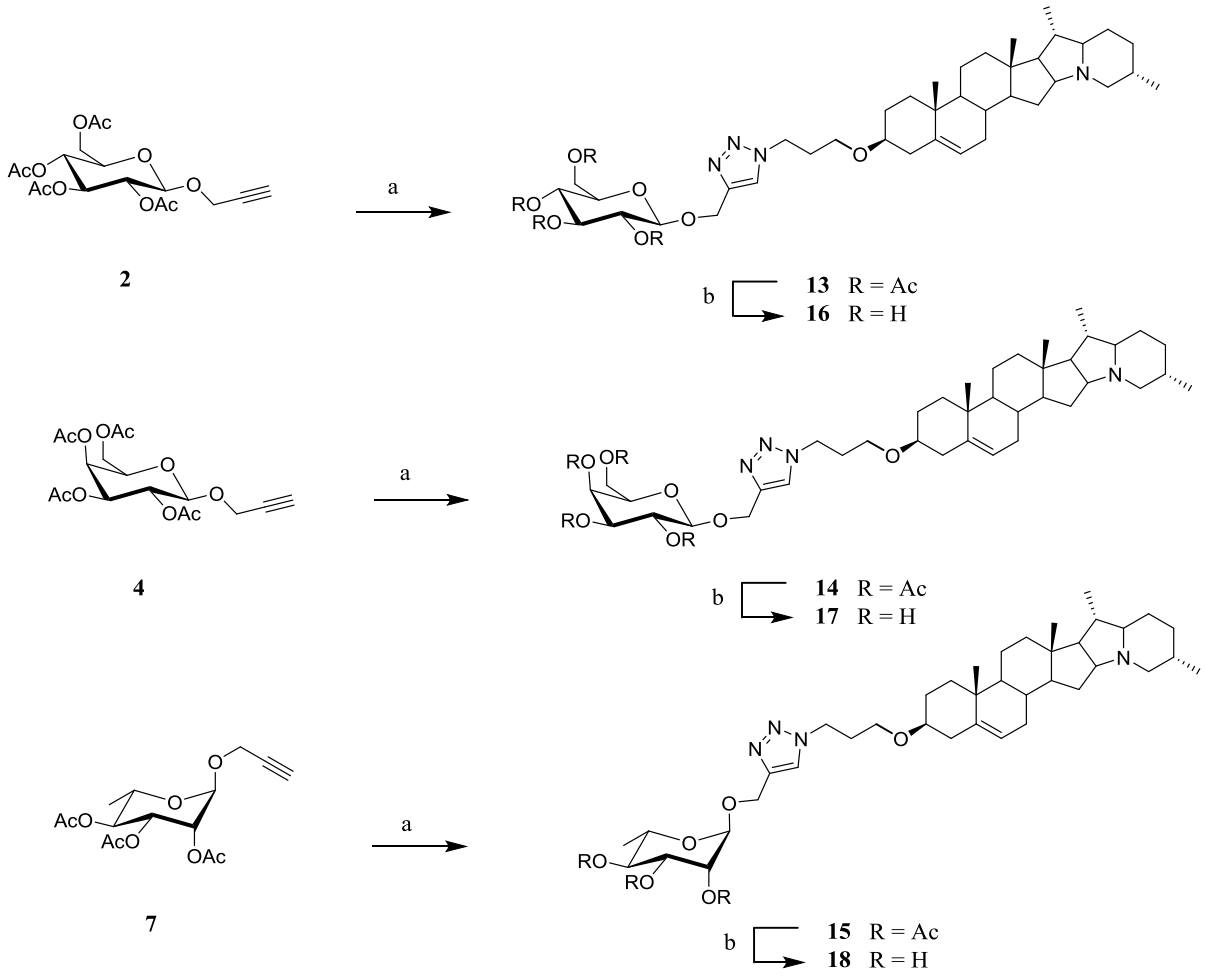

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534 Fig. 2.

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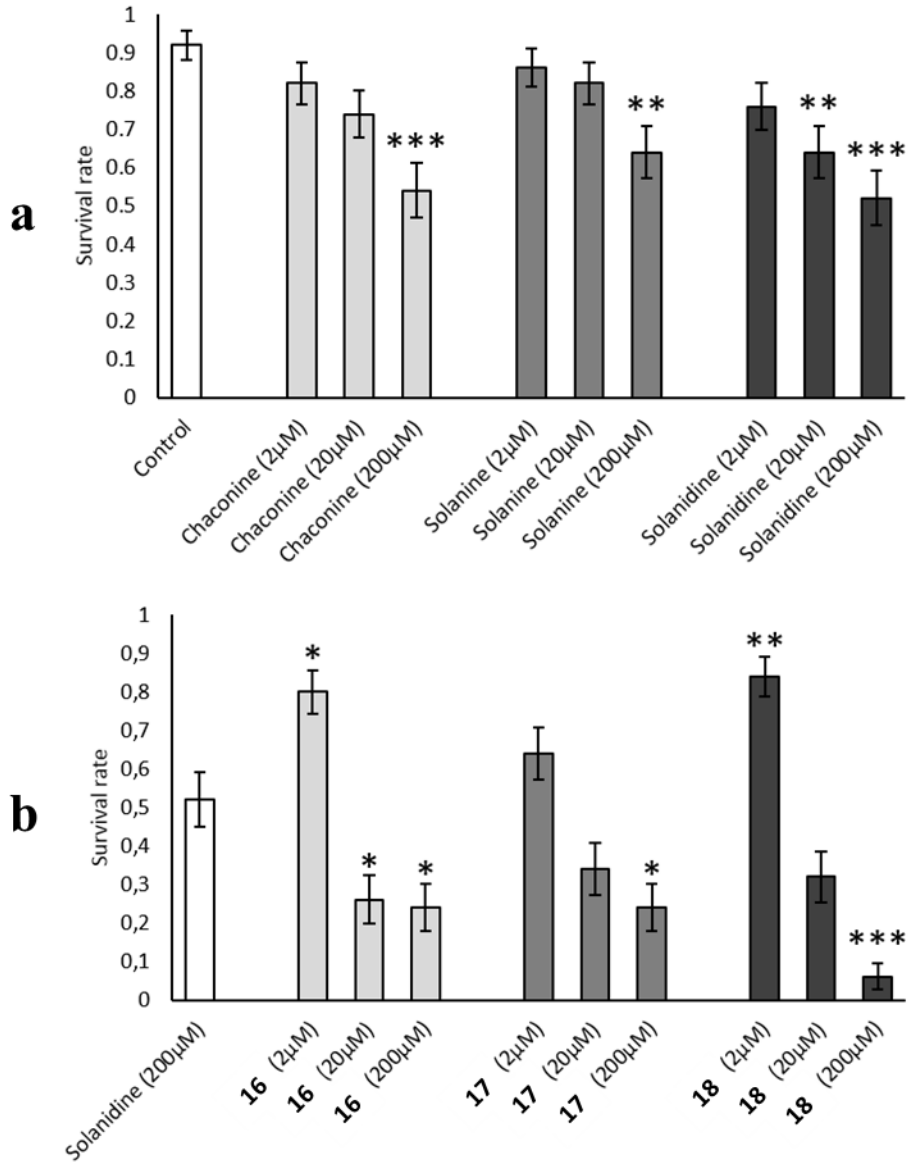
a
Survival rate
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
***
**
**
***
Control
Chaconine (2μM)
Chaconine (20μM)
Chaconine (200μM)
Solanine (2μM)
Solanine (20μM)
Solanine (200μM)
Solanidine (2μM)
Solanidine (20μM)
Solanidine (200μM)
b
Survival rate
1
0,9
0,8
0,7
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0,3
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*
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**
***
Solanidine (200μM)
16 (2μM)
16 (20μM)
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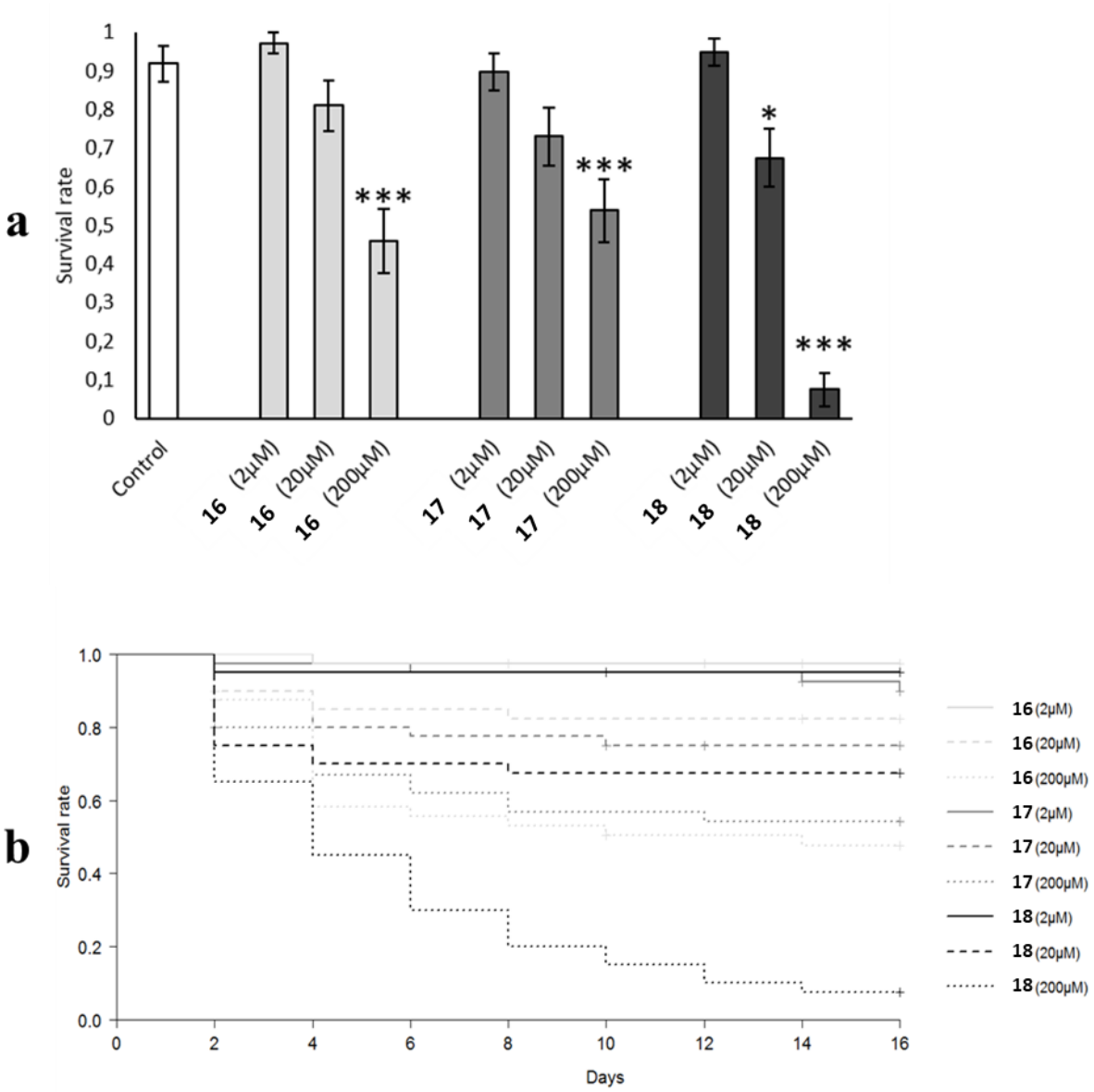
18 (20μM)
18 (200μM)

Fig. 3.



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543 Fig. 4.

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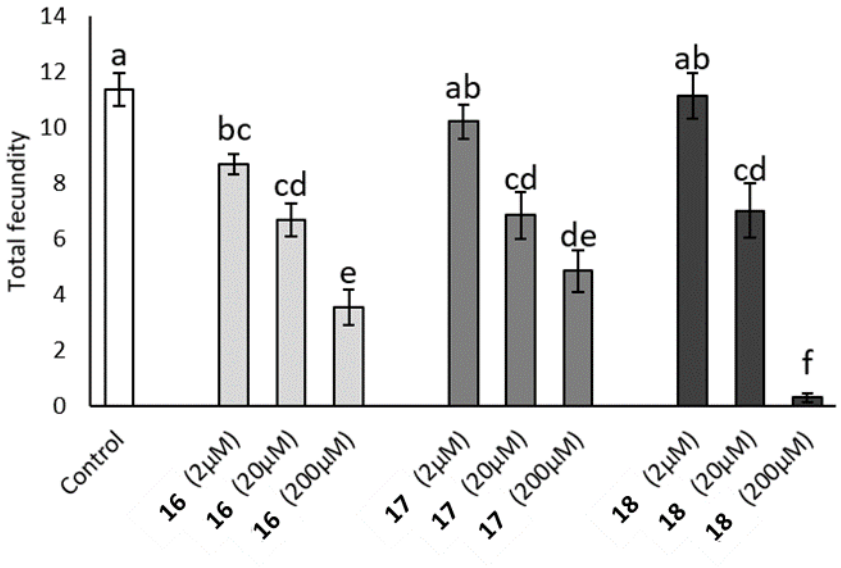

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547 Fig. 5.

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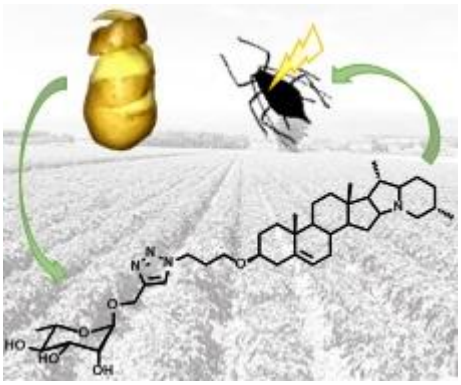
Graphical abstract



Synthesis and Insecticidal Activities of Novel Solanidine Derivatives

Rémi Beaulieu, Eric Grand, Imane Stasik, Jacques Attoumbré, Quentin Chesnais, Virginie Gobert, Arnaud Ameline, Philippe Giordanengo, José Kovensky*

This article describes the synthesis of new glycoalkaloids starting from potato solanidine. The synthetic neoglycoalkaloids showed aphicidal activities against *Macrosiphum euphorbiae* nymphs and adults.