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Strong Aphicidal Activity of GlcNAc(β1→4)Glc Disaccharides: Synthesis, Physiological Effects, and Chitinase Inhibition

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Abstract: The synthesis of four GlcNAc(β1→4)Glc disaccharides containing 2-*O*-acetyl and/or 6-sulfate groups was performed in high yields with total 1,2-*trans* stereoselectivity. These disaccharides were evaluated as candidates for insect chitinase inhibition and aphicidal activity. All the compounds prepared displayed physiological effects on *M. persicae* aphids; however, the inhibition of chitinases of different sources (bacteria, fungus, and aphid) followed different patterns according to subtle structural characteristics.

Keywords: carbohydrates · chitinases · glycosylation · inhibitors · insect

Introduction

In most insect species, chitin is the major component of the peritrophic membrane,[1,2] a non-cellular barrier formed by the digestive epithelium to compartmentalize digestive enzymes and protect epithelial cells from disruption and the penetration of parasites.[3,4] The turnover of the peritrophic membrane involves chitinases, which represent a digestive target in the strategy of chitinase inhibitors. The hemipteran *Myzus persicae* (Aphididae) is one of the most polyphagous insects worldwide, as it successfully develops on hundreds of plant species. *M. persicae*, as all homoptera, is deprived of a peritrophic membrane, and no chitinase activity has so far been described in the aphid midgut. Thus, aphids could be considered as non-digestive target pests in the context of chitinase-inhibitor use. Aphids are hemimetabolous and larviparous insects, that is, the offspring develop in the genital tracts of adults. During their embryonic development, the growth of larvae is influenced by the physiology of the fundatrix, and their integument chitinases, involved in cuticle remodelling, are possible targets. All these characteristics make *M. persicae* an interesting model to study chitinase-inhibitor effects.

We recently demonstrated the potential use of chitinase inhibitors as an aphid-management tool.[5] Differential aphicidal effects on *M. persicae* have been induced by allosamidin, a pseudotrisaccharide that is known to inhibit competitively chitinases[6,7] by mimicking chitin.

Blattner et al.,[8,9] synthesized and tested a set of allosamidin analogues to evaluate the structural requirements for chitinase inhibition. For a *Chironomus tentans* (Diptera, Chironomidae) enzyme, one *N*-acetylallosamine residue can be omitted without impairment of enzyme inhibition and glucosamine can replace allosamine in inhibitory activity without any negative effect. Similar requirements have been reported for *Tineola bisselliella* (Lepidoptera, Tineidae) and *Lucilia cuprina* (Diptera, Calliphoridae) larvae mortality,[8] and these results showed that the β-1,4 linkage between the sugar (or pseudosugar) moieties is necessary for biological activity. Moreover, these authors also demonstrated a direct correlation between larval mortality and chitinase inhibition. These findings suggest that β-1,4-*gluco* disaccharides could be appropriate candidates for insect-chitinase inhibition.

We report herein:

1) The synthesis of four GlcNAc(β1→4)Glc disaccharides. Our synthetic approach involved the introduction of a sulfate group at C6 to investigate the possible additional cooperative interactions with the protein. Sulfated oligosaccharides display a wide spectrum of biological activities, mainly through the interaction of sulfate groups with side chains of basic residues (i.e., arginine (Arg), lysine (Lys)) of proteins.[10] The role of a 2-*O*-acetyl group on the glucose unit (as a *N*-acetylglucosamine mimic) was also addressed, thus giving rise to four analogues: non-sulfated 2-*O*-acetyl **1**, 6-sulfate 2-*O*-acetyl **2**, non-sulfated 2-OH **3**, and 6-sulfate 2-OH **4** (Scheme 1).

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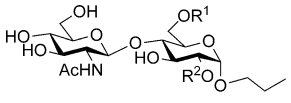


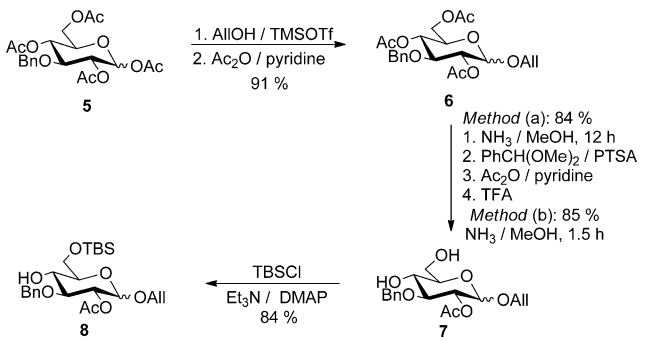
1 R^1 = H, R^2 = Ac
2 R^1 = SO_3Na, R^2 = Ac
3 R^1 = H, R^2 = H
4 R^1 = SO_3Na, R^2 = H

Scheme 1. Target disaccharides.

2) The evaluation of the physiological effects of these disaccharides on *M. persicae* aphids fed on artificial media containing different concentrations of these disaccharides.
3) The inhibition tests performed on *Streptomyces griseus* (Actinomycetales, Streptomycetaceae), *Trichoderma viride* (Hypocreales, Hypocreaceae), and *M. persicae* chitinases.

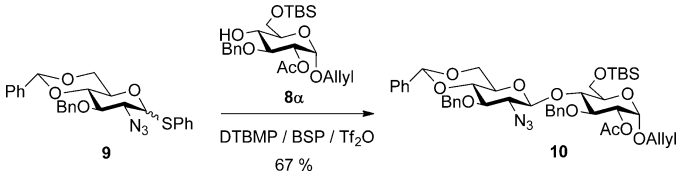
Results and Discussion

Synthesis: The synthesis of the glycosyl acceptor **8** was performed starting from the known tetracetate **5**,[11] which is easily prepared in three steps from the commercially available 1,2:5,6-di-*O*-isopropylidene-α-D-glucopyranose (Scheme 2). Compound **5** was treated with allylic alcohol in the presence of TMSOTf. No glucoside formation was detected in dichloromethane due to the low reactivity of the allylic alcohol. The use of the alcohol as a reactant and solvent allowed us to obtain the corresponding allyl glucoside at high temperature (100 °C), but these conditions led to partial deacetylation. Anyway, the glycosylation reaction followed by a reacetylation step gave the desired glucoside **6** (α/β = 3:2) in 91% overall yield. After complete deacetylation, the 4- and 6-hydroxy groups were protected by a benzylidene acetal group, the 2-hydroxy group was acetylated, and the benzylidene acetal group was hydrolysed to give the diol **7** in 84% yield over the four steps. It is interesting to note that under the conditions of the previous complete deacetylation (7 M NH_3 in MeOH) the 2-acetyl group was quite resistant. Therefore, it was possible to control this reaction to decrease the number of synthetic steps. Thus, time-controlled deacetylation of **6** allowed us to obtain **7** directly in 85% yield (Scheme 2). Compound **7** was selectively silylated at the primary hydroxy group to afford the glycosylation acceptor **8** in 84% yield (65% overall yield from **5**).



Scheme 2. Synthesis of the acceptor **8**. All = allyl, Bn = benzyl, DMAP = 4-dimethylaminopyridine , TBS = *tert*-butyldimethylsilyl , TFA = trifluoroacetic acid, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

After chromatographic separation of anomers α and β, the glycosylation reaction was performed on the major acceptor **8α**. In our first approach, we used an azido precursor of the glucosamine unit due to its stability and its easy conversion into a NHAc group in the last steps of the synthesis. When using thioglucoside **9**[12] as a donor, standard conditions of *N*-iodosuccinimide/trifluoromethanesulfonic acid or silver trifluoromethanesulfonate (AgOTf) were ineffective for coupling, but the system[13,14] of BSP/DTBMP/trifluoromethanesulfonic anhydride (Tf_2O) was successful to give the corresponding disaccharide in 67% yield as a 1:2 α/β mixture, from which the major disaccharide **10** was obtained in pure form (Scheme 3). In the ^{1}H NMR spectrum of **10**, the resonance of H1′ at $\delta = 4.66$ ppm (d, $J_{1',2'} = 7.9$ Hz) confirmed the formation of the β-linkage.

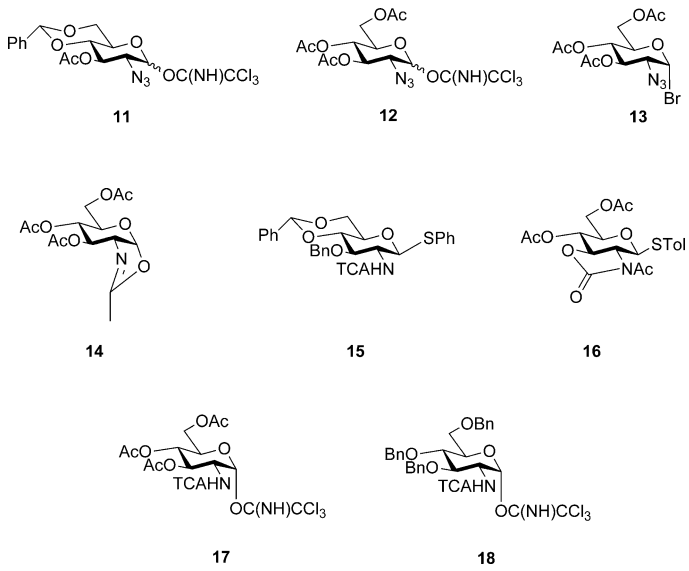


Scheme 3. Synthesis of disaccharide **10**. BSP = 1-benzenesulfinylpiperidine, DTBMP = 2,6-di-*tert*-butyl-4-methylpyridine.

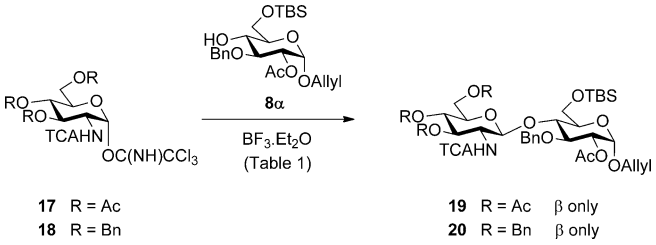
This first strategy allowed us to obtain the disaccharide precursor. However, in the key glycosylation step to give the α/β mixture, part of the product is useless and careful separation was needed to isolate the β-disaccharide. Therefore, we investigated different routes for the stereoselective formation of the 1,2-*trans* linkage. Because the 2-azide group is non-participating, the use of acetonitrile as the solvent was reported to favour 1,2-*trans* glycosylation reactions.[16] Unfortunately, the glycosylation between thioglycoside **9** and acceptor **8α** in acetonitrile failed. No coupled product was detected in spite of the use of different activation systems.

Trichloroacetimidate **11**[17] (Scheme 4) in dichloromethane in the presence of TMSOTf gave, as expected, a 1:1 α/β mixture in 50% yield. The more flexible donor **12**[18] gave similar disappointing results. The use of acetonitrile with trichloroacetimidate donors failed again to favour the formation of the desired disaccharide, only a donor rearrangement to non-glycosylating species (i.e., glycosyl trichloroacetamide) was observed when $BF_3{\cdot}Et_2O$ was used as a promoter.

On the other hand, $Sc(OTf)_3$ has been reported to give 1,2-*trans* disaccharides.[19] However, when the glycosylation of donor **12** and acceptor **8α** was performed in the presence of this catalyst, the mass spectrum of the product showed



Scheme 4. Structures of donors **11**–**18**. TCA = trichloroacetic acid, Tol = tolyl.



Scheme 5. Synthesis of disaccharides **19** and **20**.

Table 1. Stereoselective glycosylation between NHTCA-protected trichloroacetimidates and acceptor **8**α.

Entry	Donor	Promoter [equiv]	*T* [°C]	Product	Yield [%]
1	**17**	TBSOTf[a] (0.2)	−60→−20	**19**	51
2	**17**	$BF_3{\cdot}Et_2O$ (1.5)	−60→−20	**19**	61
3	**17**	$BF_3{\cdot}Et_2O$ (1.0)	−40→−20	**19**	75
4	**18**	$BF_3{\cdot}Et_2O$ (2.0)	−40→−20	**20**	61
5	**18**	$BF_3{\cdot}Et_2O$ (1.0)	−40→−20	**20**	71

[a] TBSOTf = *tert*-butyldimethylsilyl trifluoromethanesulfonate.

a molecular ion $[M+Na^+]$ of *m*/*z* 688, which corresponds to a 6-desilylated disaccharide. Moreover, in the ^{13}C NMR spectrum, the resonance for C6 appeared at $\delta = 68.2$ ppm, thus indicating that this hydroxy group was not free. Therefore, under these conditions, desilylation in situ followed by glycosylation of the more nucleophilic 6-hydroxy group was produced, thus leading to the formation of the 1→6 disaccharides.

Bromide **13**,[20] under AgOTf activation,[21] was assayed at −40 and 0 °C, thus leading to the formation of the 1→6 disaccharides as α/β mixtures. On the other hand, the use of a AgOTf/tetramethylurea system[22] did not give any coupled product, only the hydrolysed donor and the desilylated acceptor were detected in the reaction mixture.

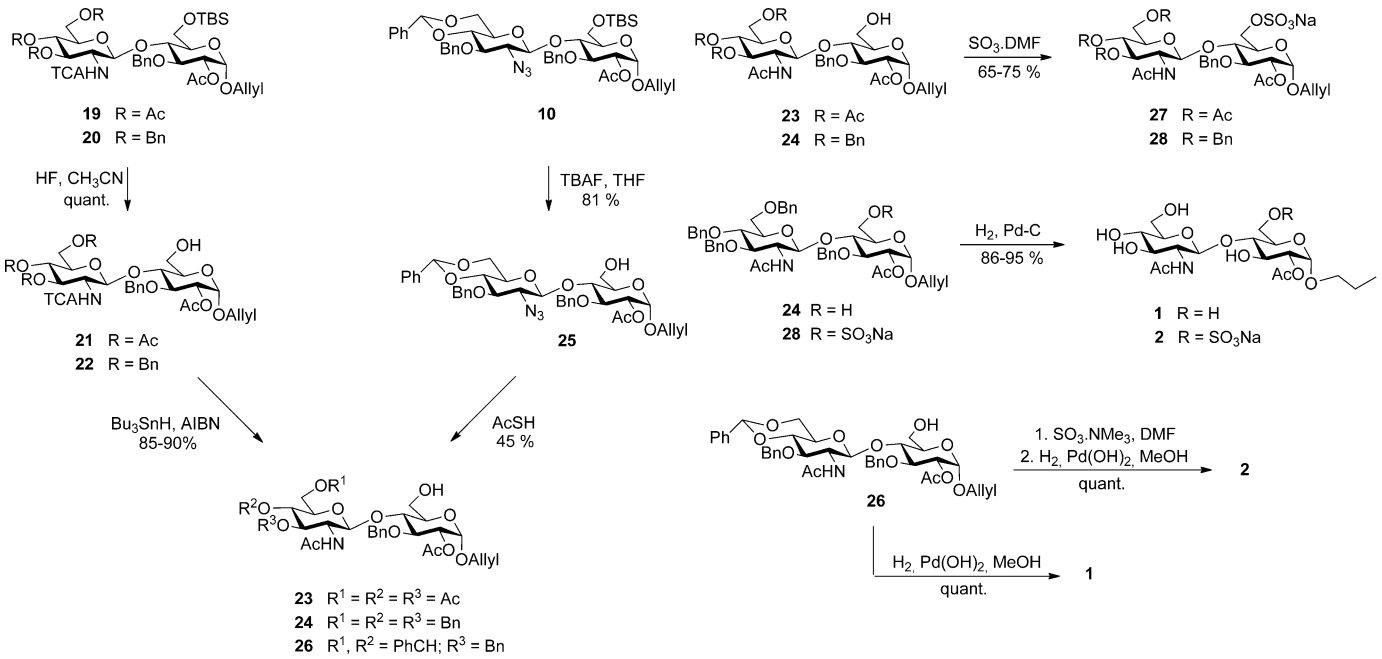
The disappointing results obtained with the use of the strong deactivating 2-azido group prompted us to investigate the use of amino-protected precursors. The use of oxazoline **14**[23] has been reported as a glycosyl donor that favours the formation of β-disaccharides.[24] However, no disaccharide formation occurred when **14** was treated with **8**α in dichloromethane in the presence of camphorsulfonic acid. The thiophenyl NHTCA donor **15**, easily obtained from **9** (β anomer) by using a two-step procedure,[25] failed to give the expected 1→4 disaccharide by using the Ph_2O/Tf_2O activating system,[26] the same by-products (desilylated acceptor and 1→6 disaccharide formation) were observed.

On the other hand, oxazolidinone **16**,[27] when reacted under BSP/2,4,6-tri-*tert*-butylphenyl (TTBP)/Tf_2O activation,[28] led stereoselectively to the (β1→4) disaccharide in 30% yield. The different changes in the glycosylation conditions did not succeed in improving the yield. Finally, trichloacetimidates **17** and **18**[29] gave the best results (Scheme 5, Table 1).

Acetylated donor **17** under TBSOTf promotion led to disaccharide **19** (51%; Table 1, entry 1). Among the by-products, we could identify the hydrolysed donor and the subsequent 1-silylated derivative, the separation of the latter and the disaccharide product was very difficult, thus lowering the yield of the recovered product. On the other hand, the use of $BF_3{\cdot}Et_2O$ allowed a little improvement of the yield (61%; Table 1, entry 2). By starting the reaction at higher temperature (−40 °C instead of −60 °C) allowed us to obtain the desired disaccharide **19** in 75% yield in the presence of 1.0 equivalents of the promoter (Table 1, entry 3). Similar conditions were applied to benzylated donor **18** (Table 1, entries 4 and 5), for which 1.0 equivalents of $BF_3{\cdot}Et_2O$ gave the highest yield of **20** (71%). The reactions were totally β stereoselective, which is shown by the coupling constants of $J_{1',2'} = 8.3$ and 7.9 Hz (for **19** and **20**, respectively).

In our preliminary tests, we showed that a sulfated disaccharide (i.e., **2**) induced differential aphicidal effects on *M. persicae*[30] and that the non-sulfated analogue (i.e., **1**) was less active. Both molecules bear a 2-*O*-acetyl substituent in the glucose unit, thus replacing (or mimicking) the NHAc group present in the chitobiose sequence. Whether this acetyl group is essential or not for the aphicidal activity is an interesting issue. Two additional disaccharides **3** and **4** were prepared to elucidate this question. By starting from the protected precursors **19** and **20**, a careful sequential deprotection strategy was designed to obtain the sulfated and non-sulfated versions of the 2-OH/2-OAc analogues and was performed in parallel.

Regioselective sulfation of the 6-position required desilylation as the first step in the deprotection sequence (Scheme 6). The classical method of tetrabutylammonium fluoride (TBAF) in THF was used for this purpose; however, partial deacetylation and acetyl migration were observed at 0 °C. The use of lower temperatures to prevent deacetylation did not lead the reaction to completion. On the other hand, the desilylation of **19** and **20** proceeded cleanly to afford **21** and **22** quantitatively by using HF in acetonitrile.



Scheme 6. Deprotection of the 6-hydroxy group and conversion into NHAc.

Scheme 7. Sulfation and final deprotection steps.

At this point it was convenient to convert the trichloroacetamido group into the final acetamido group. Hydrogenolysis on Pd/C, however, did not succeed. We observed that the two chlorine atoms were rapidly replaced by hydrogen atoms, but the last chlorine atom remained after several cycles of hydrogenolysis. Therefore, the conversion was performed using Bu_3SnH/azobisisobutyronitrile (AIBN) in toluene at reflux. Careful washing with hexane followed by purification by means of RP-18 flash chromatography ensured the absence of tin compounds in the 2-acetamido products **23** and **24**. On the other hand, starting from **10**, desilylation in TBAF/THF led to the alcohol **25** in 81% yield. The azido function was reduced by thioacetic acid,[15] thus directly giving the NHAc derivative **26** in 45% yield.

The 6-sulfation of **23** and **24** was accomplished with SO_3·DMF to give **27** and **28** in 66 and 75% yield, respectively (Scheme 7). The classical conditions, involving heating at 55°C, were adequate to yield the acetylated derivative **23**, but led to important degradation in the case of the benzylated compound **24**. Carrying out the reaction at room temperature needed longer reaction times, but prevented the formation of undesirable by-products. Targets **1** and **2** were obtained by hydrogenolysis of **24** and **28** (95 and 86% yield, respectively). Direct hydrogenation from **26** gave **1**, whereas sulfation followed by hydrogenolysis afforded **2** quantitatively.

The acetylated precursors **23** and **27** were treated with NaOMe in MeOH to give **29** and **30**, respectively, which on hydrogenolysis afforded the targets **3** (6-OH) and **4** (6-sulfate) in 80 and 76% yields, respectively, non-acetylated at O2.

Physiological effects on *Myzus persicae*: A standard diet adapted for *M. persicae* was used as a carrier for the dilution of compounds **2**–**4**, and as a control. The concentrations of the synthesized disaccharides (10 and 100 $\mu g\,mL^{-1}$) incorporated into the standard diet were chosen according to Saguez et al.[5] The *Myzus persicae* nymphal survival, pre-reproductive period (PRP; i.e., the period of time from birth until the onset of reproduction), and adult emergence were recorded every three days.[31]

Whatever the dose (i.e., 10 or 100 $\mu g\,mL^{-1}$), the three disaccharides decreased *M. persicae* nymphal survival (see Figure 1 and Table 2). Except for **3** when delivered at 10 $\mu g\,mL^{-1}$ (71.1% survival), more than 30% of the intoxicated aphids died. An increase of the dose of **2** and **4** in the artificial medium did not significantly enhance nymphal mortality, whereas an increased dose of disaccharide **3** caused more than 50% mortality at 100 $\mu g\,mL^{-1}$.

The PRP of the aphids reared in the disaccharide-complemented artificial diet was also altered relative to aphids reared in the control diet (10.6 ± 0.2). Thus, the PRPs of the aphids intoxicated with 100 $\mu g\,mL^{-1}$ of **2**, **3**, or **4** were significantly enhanced to (11.9 ± 0.3), (16.3 ± 0.9), and (15.8 ± 1.0) days, respectively. Moreover, aphids reared in 10 $\mu g\,mL^{-1}$ of **3** showed a significant enhancement of PRP to 11.9 ± 0.5 days.

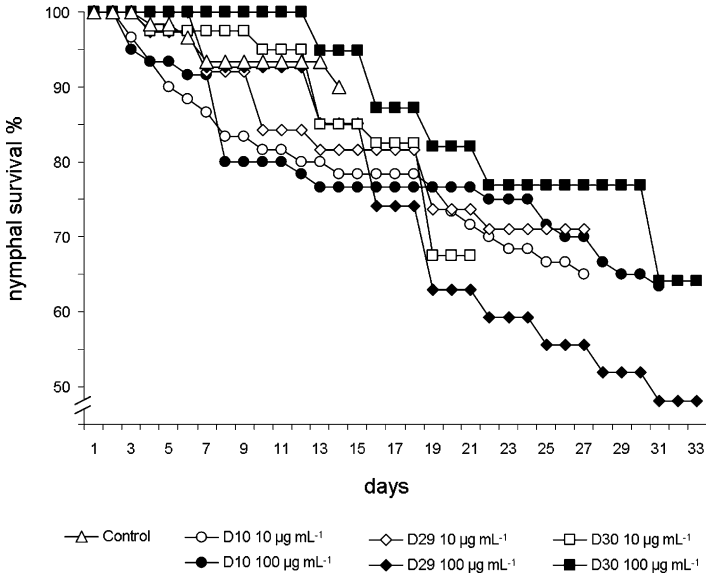


Figure 1. Nymphal survival of *Myzus persicae* reared on diets containing 0 (control), 10, or 100 $\mu g\,mL^{-1}$ of disaccharides **2** (D10), **3** (D29), or **4** (D30).

Whatever the disaccharide added to the artificial medium, adult emergence was significantly decreased relative to the control (i.e., non-intoxicated aphids; 90%). When intoxicated with disaccharide **2** at 10 and 100 $\mu g\,mL^{-1}$, as with the non-intoxicated control aphids, all the surviving nymphs became adults. At the two experimented doses, **3** and **4** significantly decreased adult emergence because 19–26% nymphs never moulted into adult.[32]

Moreover, the effects of **3** and **4** were evident on visual observation, that is, the intoxicated aphids were smaller and less coloured, especially when the sulfated disaccharide was delivered at higher doses (Figure 2).

Chitinase inhibition: Chitinase inhibition bioassays were performed on **1**–**4** (Table 3). We used two different substrates to identify exo- and endochitinase present in different extracts, that is, 4-methylumbelliferyl *N,N′*-diacetyl-β-D-chitobioside and 4-methylumbelliferyl *N,N′,N″*-triacetyl-β-D-chitotriose, respectively. Compounds **1** and **3** were active toward *S. griseus* endochitinase with $IC_{50}=175$ and 138 μM, respectively. The sulphated disaccharides **2** and **4** showed very weak inhibition activity toward bacteria chitinases with both substrates. The mode of the inhibition was determined by measuring kinetic parameters and by plotting the data in Lineweaver–Burk plots (see the Supporting Information). The results clearly showed that **1** and **3** both act as uncompetitive inhibitors that affect the V_{max} and K_m values. By using the standard reciprocal Michaelis–Menten equation for an uncompetitive inhibition, $K_i=68$ and 100 μM for **1** and **3**, respectively. Moreover, a chitinase inhibition bioassay of *T. viride* chitinases with an endochitinase substrate showed no inhibition with the sulphated and non-sulphated disaccharides. However, toward the exochitinase substrate, the disaccharide **3** exhibited a relative strong inhibition with $IC_{50}=235$ μM. The mode of the inhibition in Lineweaver–Burk plots showed that disaccharide **3** acts as a competitive inhibitor affecting K_m and not V_{max}. By using the Michaelis–Menten equation for competitive inhibition, the $K_i=82$ μM for of disaccharide **3**.

Compounds **3** and **4** showed significant inhibition toward *M. persicae* chitinases with an exochitinase substrate with $IC_{50}=123.9$ and 115 μM, respectively. The mode of the inhibition in the Lineweaver–Burk plots showed that **4** acts as a non-competitive inhibitor that affects the V_{max} and not the K_m value, although **3** acts as a competitive inhibitor that affects the K_m and not the V_{max} value. By using the Michaelis–Menten equation, $K_i=115$ and 100 μM for **3** and **4**, respectively.

Allosamidine, a chitinase inhibitor used as a positive control, showed strong inhibition toward not only the exochitinase of *T. viride* and endochitinase of *S. griseus*, but also toward the chitinase extract from *M. persicae*, with $IC_{50}=$ 8.54, 3.34, and 5.93 μM, respectively. On the other hand, Psammapline A, another chitinase inhibitor, did not show any inhibition toward all the chitinases tested.

Conclusion

A model derived from an X-ray structural analysis of allosamidin complexed to the binding cleft of hevamine,[33] a plant chitinase with a catalytic domain very similar to that of insect chitinases,[34] has been reported. The binding features in aqueous solution were studied by NMR spectroscopic analysis.[35] Allosamidin (in its protonated form) may be considered to be an analogue of the proposed positively charged reaction intermediate (oxazolinium ion) of chitin hydrolases. On the other hand, our new molecules **1**–**4** are

Table 2. Nymphal survival rates, PRP (mean ±SD), and adult-emergence rates of *Myzus persicae* reared on diets containing chitinase inhibitor **2**, **3**, or **4**.[a,b]

Compound	Control	**2**	**2**	**3**	**3**	**4**	**4**
Concentration	$n=60$	10 $\mu g\,mL^{-1}$	100 $\mu g\,mL^{-1}$	10 $\mu g\,mL^{-1}$	100 $\mu g\,mL^{-1}$	10 $\mu g\,mL^{-1}$	100 $\mu g\,mL^{-1}$
Number of larves		$n=60$	$n=60$	$n=50$	$n=50$	$n=50$	$n=50$
Nymphal survival [%]	90.0 a	65.0 b	63.3 b	71.1 b	48.1 c	67.5 b	64.1 b
PRP [days]	10.6 ± 0.2 a	10.9 ± 0.2 a	11.9 ± 0.3 b	11.9 ± 0.5 b	16.3 ± 0.9 c	11.6 ± 0.3 a	15.8 ± 1.0 c
Adult emergence [%]	90.0 a	65.0 b	63.3 bc	52.0 bd*	24.0 e*	50.0 cd*	38.0 d*

[a] Values in the same row followed by the same letter indicate that they do not differ significantly according to the chi-square test ($P<0.05$). [b] Asterisks indicate a significant difference between nymphal survival rate and adult-emergence rate for a same diet according to the chi-square test ($P<0.05$).

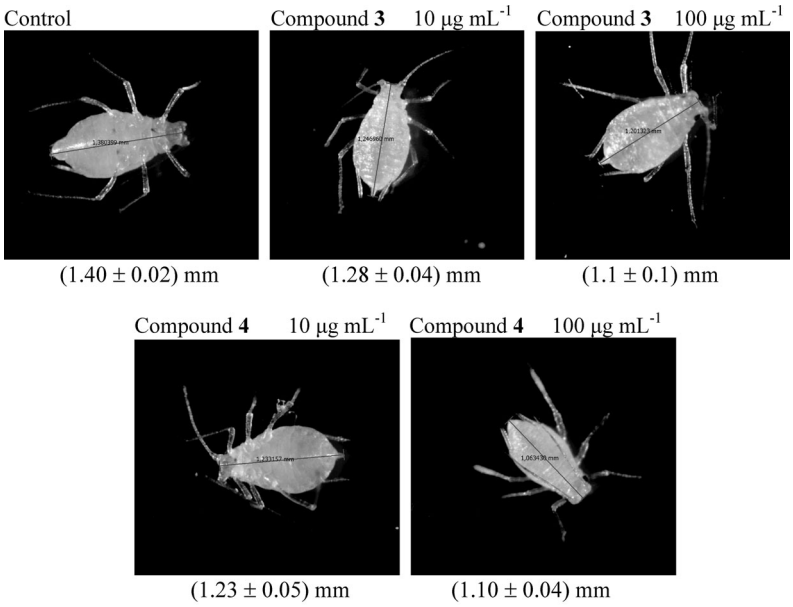


Figure 2. Photographs under stereomicroscopy of aphids fed on control diet or on diets containing disaccharide **3** or **4** at 10 or 100 μg mL^{-1}. The value is the mean of five measures.

Table 3. Inhibition assays of exo- and endochitinases of different sources.

Compound	*Streptomyces griseus*		*Trychoderma viride*		*Myzus persicae*	
	endo	*exo*	*endo*	*exo*	*endo*	*exo*
1	IC_{50} = 175 μM K_i = 68 μM uncompetitive	n.i.	n.i.	n.i.	n.i.	n.i.
2	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
3	IC_{50} = 138 μM K_i = 100 μM uncompetitive	n.i.	n.i.	IC_{50} = 235 μM K_i = 82 μM competitive	n.i.	IC_{50} = 123 μM K_i = 115 μM competitive
4	n.i.	n.i.	n.i.	n.i.	n.i.	IC_{50} = 115 μM K_i = 100 μM non-competitive
psammapline A	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
allosamidine	IC_{50} = 3.34 μM	n.i.	n.i.	IC_{50} = 8.54 μM	n.i.	IC_{50} = 5.93 μM

n.i. = no inhibition.

substrate analogues as they conserve the β-1,4 linkage and one *N*-acetyl group at the glucosamine unit.

Previous studies in the solid state[33] showed that the two *N*-methyl groups of the reducing unit of allosamidin form strong van der Waals interactions with the side chain of a Tyr6 unit at the binding site. The α-propyl chain of **3** could be involved in a similar interaction due to its orientation, as among the new synthetic compounds reported herein it is the only compound that shows competitive inhibition of the *M. persicae* exochitinase. It is clear that the negatively charged **4** cannot inhibit chitinase through the same mechanism because the bulky sulfate group may prevent binding to the catalytic site. However, this compound might interact with the positively charged sites of the protein, as suggested by the fact that this sulfated disaccharide inhibits exochitinase in a non-competitive fashion. In this study, a sulfate group was introduced at the 6-position because the primary hydroxy group can be easily differentiated, thus further developments, including sulfation at other positions, would of interest in the near future.

Surprisingly, the other sulfated disaccharide **2** did not show inhibition, in spite of the physiological effects observed in aphids, thus making it difficult to establish a clear correlation between aphicidal activity and chitinase inhibition for this molecule. In addition, when comparing the structures of the four disaccharides, the 2-*O*-acetyl of the glucose moiety is absent in the two inhibitors of the *M. persicae* exochitinase **3** and **4**, which contradicts our initial assumption that this group mimics the *N*-acetyl group of a second GlcNAc unit. On the other hand, the synthesis of the non-acetylated disaccharides is simpler because the acetyl protecting group can be used for positions −2, −3′, −4′, and −6′.

Moreover, these results suggest that the sulfate group is not essential for inhibition, and even for the aphicidal effects, in spite of the fact that the aphids seemed to be more sensitive to the sulfated molecules in our preliminary experiments.

As a consequence, the strong aphicidal effects of the new compounds observed on *M. persicae* cannot be explained exclusively on the basis of chitinase inhibition, at least for **1** and **2**. Further studies should be performed to identify other possible targets of these new disaccharides. Nevertheless, the strong physiological effects observed on *M. persicae* make these carbohydrate derivatives promising compounds for the substitution of pesticides toxic for humans or mammals in culture treatments. The fact that the four molecules are devoid of cytotoxic effects reinforces this perspective.

Experimental Section

General procedures: All the purchased materials were used without further purification. Dichloromethane and DMF were distilled from calcium hydride, pyridine over KOH, and THF over sodium and benzophenone. Analytical TLC was carried out using Merck DC-Alufolien Kieselgel 60 F_{254}. Flash chromatography was performed by using Geduran 60, 0.040–0.060 mm pore size with distilled solvents. ^{1}H and ^{13}C NMR spectra were

recorded at 300 and 75.5 MHz, respectively, on a Bruker AC300 spectrometer at 600 and 150 MHz, respectively, on a Bruker AC600 spectrometer. The chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) or a residual solvent peak ($CHCl_3$: $\delta=$ 7.26 and 77.2 ppm for ^1H and ^3C, respectively). Peak multiplicity is reported as singlet (s), doublet (d), triplet (t), quartet (q), pentet (p), multiplet (m), and broad (br). High-resolution mass spectrometry (HRMS) with electrospray ionization (ESI) was carried out on a Micromass-Waters Q-TOF Ultima Global instrument. Optical rotations were measured on a 343 PerkinElmer instrument at 208 °C in a cell (1 cm) in the stated solvent. $[\alpha]_D$ values are given in $10^{-1}\,cm^{-1}\,g^{-1}$ (concentration c given as $g\,100\,mL^{-1}$).

Synthesis of disaccharide 10: A mixture of 1-(phenylsulfinyl)piperidine (62.8 mg, 0.30 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (61.6 mg, 0.30 mmol), and molecular sieves 4 Å (400 mg) in anhydrous CH_2Cl_2 (2 mL) was stirred 30 min at room temperature in an Ar atmosphere. After cooling at −60 °C, Tf_2O (50 μL, 0.30 mmol) was added, and the stirring was continued for another 30 min. A solution of **9**[12] (111.0 mg, 0.23 mmol) in anhydrous CH_2Cl_2 (2 mL) was added, and the reaction mixture was stirred at −60 °C for 15 min. A solution of acceptor **8α** (141.6 mg, 0.30 mmol) in anhydrous CH_2Cl_2 (2 mL) was added to the reaction mixture, which was allowed to reach 10 °C in 12 h. After the addition of methanol (10 mL), the mixture was filtered through Celite and concentrated to a syrup. Flash chromatography on silica gel (EtOAc/cyclohexane 20:80) gave the disaccharide fraction (130.1 mg, 67 %, α/β= 1:2). Further purification gave pure β-disaccharide **10** as a colourless syrup. $[\alpha]_D^{20}=-68.0$ ($c=0.1$ in acetone); ^1H NMR (300 MHz, $CDCl_3$): $\delta=7.38$ (m, 15H; Ph), 5.92 (m, 1H; CH_2CH=CH_2), 5.56 (s, 1H; C*H*Ph), 5.34 (dd, $J_{gem}=1.7$, $J_{trans}=17.1$ Hz, 1H; CH_2CH=C*H*), 5.23 (dd, $J_{cis}=$ 10.1 Hz, 1H; CH_2CH=C*H*), 5.06 (d, $J_{1,2}=3.9$ Hz, 1H; H1), 4.97 (d, $J_{gem}=$ 11.1 Hz, 1H; CH_aPh), 4.93 (d, $J_{gem}=11.2$ Hz, 1H; CH_bPh), 4.84 (d, 1H; CH_aPh), 4.82 (dd, $J_{2,3}=9.6$ Hz, 1H; H2), 4.68 (d, 1H; CH_bPh), 4.66 (d, $J_{1',2'}=7.9$ Hz, 1H; H1′), 4.21 (m, 2H; H6a, C*H*CH=CH_2), 4.06 (m, 3H; H6′a, H6b, C*H*CH=CH_2), 4.05 (dd, $J_{3,4}=9.2$ Hz, 1H; H3), 3.98 (dd, $J_{3',4'}=$ 8.8 Hz, 1H; H3′), 3.88 (m, 1H; H6′b), 3.76 (m, 1H; H5′), 3.71 (dd, $J_{4',5'}=$ 9.2 Hz, 1H; H4′), 3.54 (dd, $J_{4,5}$ 10.5 Hz, 1H; H4), 3.46 (dd, $J_{2',3'}=9.2$ Hz, 1H; H2′), 3.30 (m, 1H; H5), 2.07 (s, 3H; $COCH_3$), 0.94 (s, 9H; $C(CH_3)_3$), 0.12 ppm (s, 6H; $Si(CH_3)_2$); ^{13}C NMR (75 MHz, $CDC1_3$): $\delta=170.7$ (*C*OCH_3), 139.4, 138.1, 137.5 (Ph), 134.02 (CH_2CH=CH_2), 129.5–126.4 (Ph), 118.1 (CH_2CH=CH_2), 101.6 (*C*HPh, C1′), 95.3 (C1), 82.2 (C4′), 80.0 (C3′), 79.5 (C3), 76.9 (C4), 75.7 (*C*H_2Ph), 75.4 (*C*H_2Ph), 73.4 (C2), 71.5 (C5′), 68.9 (C6′), 68.6 (*C*H_2CH=CH_2), 67.1 (C2′), 66.4 (C5), 61.6 (C6), 26.3 ($C(CH_3)_3$), 21.3 ($COCH_3$), 18.7 (*C*$(CH_3)_3$), −4.6 ppm ($Si(CH_3)_2$); HRMS (ESI): m/z calcd for $C_{44}H_{57}N_3O_{11}Si+Na^+$: 854.3660 $[M+Na]^+$; found: 854.3653.

Procedure for the glycosylation with NHTCA trichloroacetimidates: $BF_3{\cdot}OEt_2$ (55 μL, 0.43 mmol) was added to a mixture of acceptor **8α** (200 mg, 0.43 mmol), donor **17**[29] (511 mg, 0.86 mmol), and molecular sieves 4 Å (1 g) in anhydrous dichloromethane (5 mL) at −40 °C. The reaction mixture was slowly allowed to warm to −20 °C over 4 h. *N,N*-Diisopropylethylamine (213 μL) was added to the reaction mixture, which was warmed to room temperature, filtered through Celite, and concentrated to a syrup. Flash chromatography on C18 RP column (9:1 to 0:1 H_2O/CH_3CN) afforded **19** as a white solid (287 mg, 75 %). M.p. 104–106 °C; $[\alpha]_D^{20}=+32.4$ ($c=0.5$ in $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$): $\delta=7.32$ (m, 5H; Ph), 6.75 (d, $J_{NH,2'}=9.0$ Hz, 1H; N*H*), 5.85 (m, 1H; CH_2CH=CH_2), 5.27 (dd, $J_{trans}=17.2$, $J_{gem}=1.6$ Hz, 1H; CH_2CH=C*H*), 5.23 (dd, $J_{3',2'}=10.8$, $J_{3',4'}=9.4$ Hz, 1H; H3′), 5.20 (dd, $J_{cis}=10.4$ Hz, 1H; CH_2CH=C*H*), 5.13 (dd, $J_{4',5'}=9.7$ Hz, 1H; H4′), 5.01 (d, $J_{gem}=11.7$ Hz, 1H; C*H*Ph), 5.00 (d, $J_{1,2}=3.9$ Hz, 1H; H1), 4.92 (d, $J_{1',2'}=8.3$ Hz, 1H; H1′), 4.74 (dd, $J_{2,3}=9.7$ Hz, 1H; H2), 4.66 (d, 1H; C*H*Ph), 4.23 (ddd, $J_{6a',5'}=4.6$, $J_{6a',6b'}=12.3$ Hz, 1H; H6a′), 4.11 (dd, $J=5.2$ Hz, $J_{gem}=13.2$ Hz, 1H; C*H*CH=CH_2), 4.03 (dd, $J_{2',3'}=10.8$ Hz, 1H; H2′), 4.02 (dd, $J_{6b',5'}=$ 2.3 Hz, 1H; H6b′), 4.00 (dd, $J_{3,4}=9.1$ Hz, 1H; H3), 3.97 (dd, $J=5.9$ Hz, 1H; C*H*CH=CH_2), 3.96 (dd, $J_{4,5}=9.5$ Hz, 1H; H4), 3.81 (dd, $J_{6a,5}=$ 2.9 Hz, $J_{6a,6b}=12.3$ Hz, 1H; H6a), 3.80 (dd, $J_{6b,5}=1.8$ Hz, 1H; H6b), 3.62 (m, 1H; H5), 3.60 (m, 1H; H5′), 2.05–2.00 (4 s, 12H; $4\times COCH_3$), 0.95 (s, 9H; $C(CH_3)_3$), 0.13 (s, 3H; $SiCH_3$), 0.12 ppm (s, 3H; $SiCH_3$); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=170.8$, 170.5, 170.2, 169.2 ($4\times$*C*OCH_3), 161.7 (*C*$OCCl_3$), 139.1 (Ph), 133.5 (CH_2CH=CH_2), 128.1–127.3 (Ph), 117.5 (CH_2CH=CH_2), 99.7 (C1′), 94.8 (C1), 92.2 (*C*$OCCl_3$), 78.0 (C4), 76,1 (C3), 74.9 (*C*H_2Ph), 72.9 (C2), 71.9 (C5′), 71.7 (C3′), 70.8 (C5), 68.1 (OCH_2CHCH_2), 68.0 (C4′), 61.7 (C6′), 61.5 (C6), 56.6 (C2′), 25.8 (C-$(CH_3)_3$), 20.7–20.4 ($4\times COCH_3$), 18.1 (*C*$(CH_3)_3$), −5.0, −5.3 ppm ($Si(CH_3)_2$); HRMS (ESI): m/z calcd for $C_{38}H_{54}NO_{15}Cl_3Si+Na^+$: 920.2226 $[M+Na]^+$; found: 920.2238.

Disaccharide 1: This compound was obtained as a white solid (95 % yield from **24**, quantitative form **26**). M.p. 128–131 °C; $[\alpha]_D^{20}=+88.5$ ($c=$ 0.5 in MeOH); ^1H NMR (600 MHz, CD_3OD): $\delta=4.97$ (d, $J_{1,2}=3.7$ Hz, 1H; H1), 4.64 (dd, $J_{2,3}=10.1$ Hz, 1H; H2), 4.54 (d, $J_{1',2'}=8.5$ Hz, 1H; H1′), 3.98 (dd, $J_{3,4}=8.4$ Hz, 1H; H3), 3.94 (dd, $J_{6a',5'}=2.2$ Hz, $J_{6'a,6'b}=$ 11.8 Hz, 1H; H6a′), 3.77 (dd, $J_{6a,6b}=10.0$ Hz, 1H; H6a), 3.73 (dd, $J_{2',3'}=$ 10.4 Hz, 1H; H2′), 3.67 (m, 4H; H6b, H5′, H6′b, C*H*CH_2CH_3), 3.59 (dd, $J_{4,5}=9.7$ Hz, 1H; H4), 3.49 (dd, $J_{3',4'}=8.6$ Hz, 1H; H3′), 3.67 (m, 1H; H5), 3.36 (dt, $J_{gem}=9.7$ Hz, $J=6.5$ Hz, 1H; C*H*CH_2CH_3), 3.34 (dd, $J_{4',5'}=$ 9.8 Hz, 1H; H4′), 2.10, 2.04 (2 s, 6H; $2\times COCH_3$), 1.62 (m, 2H; $CH_2CH_2CH_3$), 0.96 ppm (t, $J=7.4$ Hz, 3H; $CH_2CH_2CH_3$); ^{13}C NMR (75 MHz, CD_3OD): $\delta=173.8$, 172.4 ($2\times COCH_3$), 103.0 (C1′), 96.8 (C1), 81.4 (C4), 78.1 (C5′), 75.7 (C3′), 74.4 (C2), 72.0 (C4′), 71.7 (C5), 71.0 (C3), 70.7 ($CH_2CH_2CH_3$), 62.6 (C6′), 61.6 (C6), 57.4 (C2′), 23.6 ($CH_2CH_2CH_3$), 23.1, 20.8 ($2\times COCH_3$), 10.9 ppm ($CH_2CH_2CH_3$); HRMS (ESI): m/z calcd for $C_{19}H_{33}NO_{12}+Na^+$: 490.1900 $[M+Na]^+$; found: 490.1896.

Disaccharide 2: This compound was obtained as a white solid (86 % yield from **28**, quantitative from **26**). M.p. 191–193 °C; [α]D20= +68.5 ($c=0.5$ in MeOH); ^1H NMR (600 MHz, CD_3OD): $\delta=4.97$ (d, $J_{1,2}=$ 3.7 Hz, 1H; H1), 4.67 (dd, $J_{2,3}=10.0$ Hz, 1H; H2), 4.65 (d, $J_{1',2'}=8.4$ Hz, 1H; H1′), 4.29 (dd, $J_{6a,5}=3.2$ Hz, $J_{6a,6b}=10.9$ Hz, 1H; H6a), 4.14 (dd, $J_{6b,5}=2.0$ Hz, 1H; H6b), 3.95 (dd, $J_{3,4}=8.7$ Hz, 1H; H3), 3.95 (dd, $J_{6a',5'}=$ 2.3 Hz, $J_{6a,6b}=11.9$ Hz, 1H; H6a′), 3.86 (dd, $J_{2',3'}=10.1$ Hz, 1H; H2′), 3.84 (m, 1H; H5), 3.72 (dd, $J_{6b',5'}=6.1$ Hz, 1H; H6b′), 3.71 (dd, $J_{4,5}=9.8$ Hz, 1H; H4), 3.69 (dt, $J=6.5$, $J_{gem}=9.8$ Hz, 1H; C*H*CH_2CH_3), 3.45 (dd, $J_{3',4'}=$ 8.6 Hz, 1H; H3′), 3.46 (m, 1H; H5′), 3.39 (dd, $J_{4',5'}=9.5$ Hz, 1H; H4′), 3.38 (dt, $J=6.5$ Hz, 1H; C*H*CH_2CH_3), 2.12, 2.11 (2 s, 6H; $2\times COCH_3$), 1.64 (m, 2H; $CH_2CH_2CH_3$), 0.97 ppm (t, $J=7.4$ Hz, 3H; $CH_2CH_2CH_3$); ^{13}C NMR (75 MHz, CD_3OD): $\delta=174.1$, 172.3 ($2\times COCH_3$), 103.0 (C1′), 96.9 (C1), 81.2 (C4), 78.1 (C5′), 76.4 (C3′), 74.5 (C2), 71.8 (C4′), 70.9 (C3), 70.8 ($CH_2CH_2CH_3$), 69.6 (C5), 66.6 (C6), 62.4 (C6′), 56.8 (C2′), 23.6 ($CH_2CH_2CH_3$), 23.1, 20.7 ($2\times COCH_3$), 10.8 ppm ($CH_2CH_2CH_3$); HRMS (ESI): m/z calcd for $C_{19}H_{32}NO_{15}S^-$: 546.1498 $[M]^-$; found: 546.1493.

Disaccharide 3: This compound was obtained as a white solid (80 % yield from **29**). M.p. 129–132 °C; $[\alpha]_D^{20}=+76.6$ ($c=0.5$ in MeOH); ^1H NMR (CD_3OD, 600 MHz): $\delta=4.78$ (d, $J_{1,2}=3.8$ Hz, 1H; H1), 4.51 (d, $J_{1',2'}=8.5$ Hz, 1H; H1′), 3.93 (dd, $J_{6a',5'}=2.1$ Hz, $J_{6a',6b'}=11.8$ Hz, 1H; H6a′), 3.78 (dd, $J_{3,4}=8.7$ Hz, 1H; H3), 3.75 (m, 1H; H6a), 3.71 (dd, $J_{2',3'}=$ 10.3 Hz, 1H; H2′), 3.67 (dd, $J_{6b',5'}=6.5$ Hz, 1H; H6b′), 3.66 (dt, $J_{gem}=9.5$, $J=7.0$ Hz, 1H; C*H*CH_2CH_3), 3.63 (dd, $J_{6b,5}=4.5$, $J_{6b,6a}=11.6$ Hz, 1H; H6b), 3.61 (m, 1H; H5), 3.48 (dd, $J_{3',4'}=8.3$ Hz, 1H; H3′), 3.46 (dd, $J_{4,5}=$ 9.5 Hz, 1H; H4), 3.44 (dd, $J_{2,3}=9.8$ Hz, 1H; H2), 3.42 (dt, $J=6.5$ Hz, 1H; C*H*CH_2CH_3), 3.37 (m, 1H; H5′), 3.35 (dd, $J_{4',5'}=10.0$ Hz, 1H; H4′), 2.03 (s, 3H; $COCH_3$), 1.66 (m, 2H; $CH_2CH_2CH_3$), 0.96 ppm (t, $J=7.4$ Hz, 3H; $CH_2CH_2CH_3$); ^{13}C NMR (75 MHz, CD_3OD): $\delta=173.8$ (*C*OCH_3), 103.1 (C1′), 99.6 (C1), 81.8 (C4), 78.0 (C5′), 75.7 (C3′), 73.5 (C3), 73.0 (C2), 71.9 (C4′), 71.8 (C5), 70.8 (*C*$H_2CH_2CH_3$), 62.5 (C6′), 61.6 (C6), 57.4 (C2′), 23.7 ($CH_2CH_2CH_3$), 23.1 ($COCH_3$), 11.0 ppm ($CH_2CH_2CH_3$); HRMS (ESI): m/z calcd for $C_{17}H_{31}NO_{11}+Na^+$: 448.1795 $[M+Na]^+$; found: 448.1809.

Disaccharide 4: This compound was obtained as a white solid (70 % yield from **30**). M.p. m.p. 197–200 °C; $[\alpha]_D^{20}=+60.6$ ($c=0.5$ in MeOH); ^1H NMR (600 MHz, CD_3OD): $\delta=4.77$ (d, $J_{1,2}=3.8$ Hz, 1H; H1), 4.61 (d, $J_{1',2'}=8.8$ Hz, 1H; H1′), 4.27 (dd, $J_{6a,5}=3.0$ Hz, $J_{6a,6b}=10.8$ Hz, 1H; H6a), 4.10 (dd, $J_{6b,5}=1.9$ Hz, 1H; H6b), 3.93 (dd, $J_{6a',5'}=2.1$, $J_{6a',6b'}=11.9$ Hz, 1H; H6a′), 3.84 (dd, $J_{2',3'}=9.5$ Hz, 1H; H2′), 3.78 (m, 1H; H5), 3.75 (dd, $J_{3,4}=9.4$ Hz, 1H; H3), 3.71 (dd, $J_{6b',5'}=5.7$ Hz, 1H; H6b′), 3.66 (dt, $J=$ 6.9 Hz, $J_{gem}=9.6$ Hz, 1H; C*H*CH_2CH_3), 3.59 (dd, $J_{4,5}=9.5$ Hz, 1H; H4), 3.47 (dd, $J_{2,3}=9.7$ Hz, 1H; H2), 3.43 (dd, $J_{3',4'}=8.5$ Hz, 1H; H3′), 3.42 (m, 1H; H5′), 3.41 (m, 1H; C*H*CH_2CH_3), 3.38 (dd, $J_{4',5'}=9.3$ Hz, 1H; H4′),

2.09 (s, 3H; COCH_3), 1.66 (m, 2H; $CH_2CH_2CH_3$), 0.97 ppm (t, $J=7.4$ Hz, 3H; $CH_2CH_2CH_3$); ^{13}C NMR (75 MHz, CD_3OD): $\delta=174.1$ ($COCH_3$),102.9 (C1′), 99.7 (C1), 81.4 (C4), 78.0 (C5′), 76.4 (C3′), 73.1 (C2, C3), 71.7 (C4′), 71.1 ($CH_2CH_2CH_3$), 69.7 (C5), 66.7 (C6), 62.3 (C6′), 56.7 (C2′), 23.6 ($CH_2CH_2CH_3$), 23.1 ($COCH_3$), 10.9 ppm ($CH_2CH_2CH_3$); HRMS (ESI): m/z calcd for $C_{17}H_{30}NO_{14}S^-$: 504.1387 $[M]^-$; found: 504.1389.

Insects and feeding assays: *Myzus persicae* was reared on potato plants (*Solanum tuberosum* cv Désirée) maintained at (20±1)°C, (60±5)% relative humidity, and under a long-day (LD) photoperiod of 16:8 h. The colony was initiated from a single virginiparous female collected in early summer 1999 from a potato field near Loos-en-Gohelle (France; 50○27_27_N, 2○47_30_E). Pouches of each diet (0 (control), 10, and 100 μg mL^{-1} chitinase inhibitor) with 10–12 nymphs younger than 24 h were maintained under parthenogenesis, thus inducing conditions as described above. At least five replicates were carried out for each diet, and the pouches were changed every second day. Nymphal survival, PRP, and adult emergence were recorded every 3 days.[32]

Enzyme assays: The effects of synthetic disaccharides on the activity of chitinases from *Streptomyces griseus*, *Trichoderma viride*, or crude *Myzus persicae* extracts were analysed by using the synthetic substrate 4-methylumbelliferyl β-D-*N*,*N*-diacetylchitobioside (4-MU-$(GlcNAc)_2$) and 4-methylumbelliferyl β-D-*N*,*N*-triacetylchitotriose for exo- and endochitinase, respectively. These substrates permit accurate determination of kinetic parameters. In the assay, the reaction mixture (100 μL) contained 4 μg of the enzyme with various amounts of **1**, **2**, **3**, or **4**, Psammapline A, and Allosamidine in 50 mM citrate phosphate buffer (pH 5). The reaction mixture was incubated for 10 min at 37°C before adding the appropriate substrate concentrations and then incubated for a further 30 min at 37°C. The reaction was quenched with Na_2CO_3 (200 μL, 20 mM). The amount of 4-methylumbelliferone (4-MU) released was determined by using a multiplate fluorimeter reader (Tecan) and Magellan data analysis software. A standard curve was calculated by using five standard samples of 4-MU of concentrations between 10 and 1000 ng. The IC_{50} value was determined in a standard assay. The kinetic parameters were determined with substrate concentrations of 5, 10, 15, and 20 μM. The velocities for the kinetic parameters were calculated from the chitinase samples according the standard curve, final volume reaction, and the reaction time. The data sets were analysed by using Lineweaver–Burk plots.

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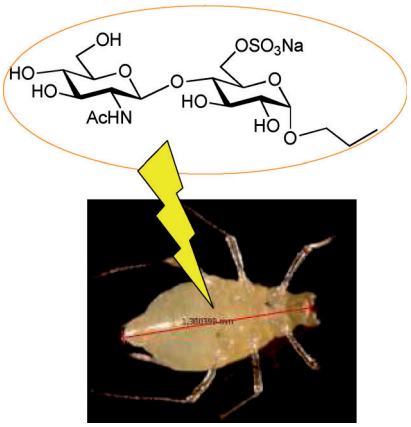
Sugar-based insecticides: Four GlcNAc(β1→4)Glc disaccharides containing acetyl and/or sulfate groups were synthesized in high yields with 1,2-*trans* stereoselectivity. These compounds are candidates for insect chitinase inhibition and aphicidal activity (see figure).



Carbohydrates

C. Dussouy, L. Bultel, J. Saguez, A. Cherqui, M. Khelifa, E. Grand, P. Giordanengo, J. Kovensky** ..... ▮▮▮▮–▮▮▮▮

Strong Aphicidal Activity of GlcNAc-(β1→4)Glc Disaccharides: Synthesis, Physiological Effects, and Chitinase Inhibition