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Synthesis of sugars embodying conjugated carbonyl systems and related triazole derivatives from carboxymethyl glycoside lactones. Evaluation of their antimicrobial activity and toxicity

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ABSTRACT

The synthesis of a series of pyranoid derivatives comprising a conjugated carbonyl function and related triazole derivatives, structurally suitable for bioactivity evaluation, was achieved in few steps starting from readily available carboxymethyl glycoside lactones (CMGL), 3-Enopyranosid-2-uloses were generated by oxidation/elimination of tri-O-acylated 2-hydroxy pyranosides. Subsequent Wittig olefination provided stereoselectively 2-C-branched-chain conjugated dienepyranosides with (E)-configuration around the exocyclic double bond. A heterogeneous Cul/Amberlyst-catalyzed 'click' chemistry protocol was used to convert glycosides bearing a propargyl moiety into the corresponding 1,2,3-triazoles. These new molecules were screened for their in vitro antibacterial and antifungal activities and those containing conjugated carbonyl systems demonstrated the best efficacy. (N-Dodecylcarbamoyl)methyl enone glycerosides were the most active ones among the enones tested. The α -anomer displayed very strong activities against Bacillus cereus and Bacillus subtilis and strong activity toward Enterococcus faecalis and the fungal pathogen *Penicillium aurantiogriseum*. The corresponding β -anomer presented a very strong inhibitory effect against two fungal species (Aspergillus niger and P. aurantiogriseum). (N-Dodecyl-/Npropargyl/or N-benzylcarbamoyl)methyl dienepyranosides exhibited selectively a strong activity toward E. faecalis. Further acute toxicity evaluation indicated low toxic effect of the (N-dodecylcarbamoyl)methyl enone glyceroside α -anomer and of the carbamoylmethyl dienepyranosides N-protected with propargyl or benzyl groups.

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1. Introduction

α,β-Unsaturated carbonyl compounds occupy a prominent place among various classes of molecular targets due to their broad spectrum of biological and pharmacological activities.¹ Their conjugated functionality, which is prompted to Michael-type addition, may represent a receptor site for bionucleophiles, notably enzyme sulfhydryl groups,² and therefore makes them suitable compounds for bioactivity screening. In particular, the incorporation of conjugated carbonyl functions in carbohydrates has led to useful substrates for derivatization in view of their ability to undergo a variety of transformations.³ Moreover, some of these sugar derivatives have shown significant biological properties. Among them, furanose C–C-linked α,β-unsaturated lactones displayed antifungal⁴ and insecticidal effects.⁵ These findings have motivated the development of synthetic methodologies towards bicyclic fused derivatives⁶ and thiosugar analogues.⁷ Sugar enones have also proven to be versatile building blocks for a variety of natural products and relevant chiral molecules, such as branched-chain sugars, *C*-glycosyl compounds or disaccharides.³ One of the most well-explored enone scaffolds in terms of synthetic uses is levoglucosenone, a 1,6-anhydro-3-enopyran-2-ulose, which is accessible by pyrolysis of cellulose.⁸ A few examples of naturally occurring enone-containing sugars have been reported, and those include the fungal metabolites Microthecin,⁹ a 3-enopyran-2-ulose which exhibited antibacterial and cytotoxic effects, and Ascopyrone P,¹⁰ a 1-enopyran-3-ulose known to possess antioxidant and antibacterial activities.

A number of methods for the synthesis of pyranoid enones have appeared in the literature,³ and some of them employ easily available glycals as starting materials. Oxidation of unprotected glycals, for example, with PDC^{11} or $Pd(OAc)_2$,¹² and of their 3-O-protected

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derivatives, using hypervalent iodine reagents,¹³ leads to 1-enopyran-3-uloses. Hex-3-enopyran-2-uloses comprise stereogenic centers next to the conjugated system which may induce stereoselectivity in addition reactions. Enones of this type can be prepared from 2-acyloxyglycal esters by simple transformations, such as chlorination followed by elimination,¹⁴ peroxidation and subsequent acetylation,¹⁵ or glycosylation of alcohols with these glycal donors in the presence of a Lewis acid.¹⁶ Nevertheless, methods that involve β-acylated hexopyranosuloses as intermediates are preferred since these compounds are prone to undergo β-elimination of acyloxy groups.¹⁷ This tendency for elimination appears to be general and has given rise either to the formation of 1-enopyran-3-uloses, from easily available furan-3-uloses, ^{7,17d} or of 3-enopyran-2-uloses from the corresponding acylated pyran-2uloses.^{17a-c} However methodologies for the synthesis of 2-uloses, which require partially acylated 2-hydroxy pyranosyl precursors. are not straightforward, involving protection and deprotection steps and low global yields. This issue can be overcome starting from carboxymethyl glycoside lactones (CMGLs).¹⁸ The opening of the lactone moiety of these compounds, especially by amines, has enabled the acquisition of various pseudo glycoconjugates, such as pseudo glycoamino acids,^{18a} pseudo disaccharides,¹⁹ nucle-otide sugars,¹⁹ pseudo glycolipids,²⁰ or more recently new glycoprobes for membranes nonlinear imaging.²¹ Moreover, the connection of the sugar to another molecular block by this strategy provides adducts containing a free and unique hydroxyl group at position 2, available for functionalization.^{18c}

Thus, we were motivated to explore the feasibility of the oxidation at C-2 of these adducts as concomitant 3,4-elimination would be expected, giving the target 3-enopyranosid-2-uloses. Tri-Oacylated 2-hydroxy gluco- and galactopyranosides, which differ in the configuration at C-1, were synthesized and different oxidation methods were used in order to investigate the influence of these factors on the efficiency of the oxidation-elimination process. Variations on the nature of the aglycon moiety also widened the panel of compounds for subsequent biological activity evaluation. Branching at C-2 of the α -enulosides by Wittig olefination was further accomplished leading to conjugated dienepyranosides. (N-Propargylcarbamoyl)methyl glycosides were used for the inclusion of an additional triazole motif, an heterocycle commonly related to a variety of bioactivities, and pyranoid derivatives comprising both the triazole and the conjugated carbonyl system were synthesized. The structural diversity of these highly functionalized pyranosidic derivatives allowed a rational study of their antimicrobial activities. Compounds' acute cytotoxicity in eukaryotic cells was also performed. In this paper both the synthetic work and the results of the biological assays are presented and discussed.

2. Results and discussion

2.1. Chemistry

2.1.1. 3-Enopyranosid-2-uloses

The α -gluco CMGL **1** was synthesized following the reported procedure based on isomaltulose oxidation followed by acetylation.^{18a} The bicyclic lactones having α -galacto (**2**) and β -gluco configuration (**3**) were prepared by anomeric alkylation of glucose or 2,3,4,6-tetra-*O*-acetyl- α -*D*-galactopyranose with *tert*-butyl bromoacetate and successive ester cleavage and cyclization.^{18c} A series of primary amines were then employed for the lactone ring-opening of CMGLs **1–3** (Scheme 1). Propargylamine was chosen aiming to a further derivatization of the terminal triple bond whereas benzylamine and dodecylamine were selected for the connection of a hydrophobic portion to the carbohydrate moiety, in order to investigate their effect in terms of antimicrobial action. The expected amides **4–10** were obtained in 66–94% vields, simply by treatment of 1-3 with the amines in dichloromethane at room temperature. Having a free hydroxyl group at C-2, the oxidation/ elimination of these adducts was subsequently exploited. The preliminary experiments were carried out with (N-propargylcarbamoyl)methyl α -glycosides. For both α -gluco (**4**) and α -galacto (**10**) derivatives, different oxidizing agents and conditions were used in the search of the most appropriate method (Table 1). The system DMSO-acetic anhydride proved to be the most effective one, giving the desired 3-enopyranosid-2-ulose 13 in better yield (ca. 60%) and as the single product. In its ¹³C NMR spectrum, the carbonyl group was observed at δ 181.7, characteristic of an α , β -unsaturated ketone. Diagnostic signals in the ¹H NMR spectra were the olefinic proton (H-4), observed at δ 6.61 as a doublet with a coupling constant I_{45} = 1.9 Hz, suggesting the adoption of an ^OE envelope (sofa) conformation.^{17b} and H-1, which was assigned at δ 4.99 as a singlet. Moreover, no significant differences in yields were obtained starting from each of the epimers suggesting that configuration at C-4 does not play a crucial role in the reaction outcome.

3,4,6-Tri-O-acetyl-glycopyranosid-2-uloses (**11** and **12**) could be detected in the oxidation of **4**/**7** with PDC/Ac₂O, and were the major products when using the Dess–Martin periodinane as milder oxidizing agent. However, isolation of the 2-ulosides was not possible by column chromatography probably due to their facile conversion to the pyranoid enone system.

The DMSO/Ac₂O procedure was thus chosen for the oxidation/ elimination of the other glycosides. Hence the α -glucosides bearing benzylcarbamoyl (**5**) and dodecylcarbamoyl moieties (**6**) were converted into the corresponding enones **14–15** in nearly quantitative yields.

The effect of the anomeric configuration was then examined. Performed under same conditions as those used previously, the DMSO/Ac₂O oxidation of the 2-hydroxy carbamoylmethyl β -glycosides **8–10** led to β -3-enopyranosid-2-uloses **16–18**, although in lower yields (32–45%) than those obtained for their α -counterparts and with some tendency to undergo decomposition. The results obtained reflect the lower stability of the products formed, due to the adopted $E_{\rm O}$ envelope conformation, indicated by the values of $J_{4,5}$ (3.3 Hz), involving energetically unfavorable 1,3-diaxial interactions between the substituent at C-1 and the acetoxymethyl group.

2.1.2. 2-C-Branched-chain conjugated dienepyranosides

Generation of 2-C-branched-chain sugars from 3-enopyranosid-2-uloses was subsequently explored. Oxidation of (N-allylcarbamoyl)methyl α -glucopyranoside **19**^{18c} with DMSO/Ac₂O to enone 20 was followed by Wittig-type treatment with the stabilized ylide [(ethoxycarbonyl)methylene]triphenylphosphorane in chloroform to provide 21 as the single product (Scheme 2). Since no other diastereomer was obtained, which would be helpful for comparison purposes, the (E)-configuration around the double bond for 21 was assigned on the basis of its ¹H NMR shift for H-1, which appeared 1.3 ppm downfield from the corresponding signal in 20, and on NOE experiments, which showed a strong correlation between H-2' and CH₃ (Ac-3) protons. This suggested the given orientation for the ethoxycarbonyl group. Similarly, Wittig olefination of 3-enopyranosid-2-uloses 13-15 furnished conjugated dienepyranosides 22-24 in moderate yields (54-61%), presenting identical ¹H NMR features as those observed for **21**, in accord with the proposed (*E*)-configuration. While a few examples of olefination reactions of pyranoid enones have appeared in the literature,²² to the best of our knowledge there was only one report involving a 3-enopyranoside-2-ulose.^{22a} These pyranoid $\alpha, \beta, \gamma, \delta$ -unsaturated esters are not only interesting for bioactivity studies but may also constitute original



12 α-galacto

Scheme 1. Reagents and conditions: (a) RNH₂, CH₂Cl₂, rt, 16 h, 66-94%; (b) see Table 1; (c) DMSO/Ac₂O, rt, 16 h, 96-99% (14, 15), 32-45% (16-18).

Table 1

Results of the oxidation of (*N*-propargylcarbamoyl)methyl 3,4,6-tri-O-acetylglycopyranosides **4** and **10**

Oxidation method and conditions	Yield for 13 ^a		
	4 (gluco)→ 13	7 (galacto)→ 13	
DMSO/Ac ₂ O, rt, 16 h PDC/Ac ₂ O, CH ₂ Cl ₂ , reflux, 1 h Dess-Martin periodinane, CH ₂ Cl ₂ , rt, 16 h	61 28 (15) 6 (15)	59 35 (5) 6 (22)	

^a Yield for the obtained 2-uloside (11 and 12) indicated in parenthesis.

templates for further synthetic elaboration through their activated diene system.

2.1.3. Triazole derivatives

Glycosides containing alkynyl moieties were engaged in Cu(I)catalyzed Huisgen 1,3-dipolar cycloadditions with a terminal azide.²³ aiming at the insertion of a 1,2,3-triazole ring which is generally recognized as one of the most biologically active motifs among heterocyclic nucleus.²⁴ Various examples of this so-called 'click' chemistry employing carbohydrates have been reported, namely in polysaccharide modification²⁵ or in the synthesis of carbohydrate macrocycles,²⁶ oligosaccharides,²⁷ and glycoconjugates,²⁸

in which the triazole unit may serve as a linker between saccharide moieties, or a spacer entity for the generation of glycopeptide mimics. We have applied a mild and simple catalytic procedure consisting on the use of copper(I) iodide supported on Amberlyst A-21 resin and dichloromethane as solvent.²⁹ The resin is a polystyrene-based polymer containing a dimethylamine group which may serve both as a chelating agent for the cooper salt and as a base.²⁹ The first attempted cycloaddition reactions between the galactoside **7** and benzyl or hexenyl azide³⁰ (Scheme 3) were completed after overnight stirring at room temperature and afforded the corresponding 1,2,3-triazole derivatives 25 and 26 in good isolated yields (70-74%). Similar conditions for the coupling of the α - and β -glucosides **4** and **8** with benzyl azide gave successfully the expected triazoles 28 and 29. Furthermore, 'click' reactions that lead to 26 or 29 afforded a small amount (4%) of a secondary product, identified by NMR and HRMS as the 5-iodo-1,2,3-triazole derivatives 27 or 30, respectively. 5-Iodo triazoles have already been reported as minor products in CuI-catalyzed alkyne-azide cycloadditions when organic bases such as NEt₃ or DIPEA (diisopropylethylamine), were used. Their formation may be due to I₂ contamination in Cul, acting as source of I^{+.31} The role of the base and the influence of its nature in facilitating the formation of 5-iodo triazoles, was recently investigated and a mechanism involving the stabilization of



Scheme 2. Reagents and conditions: (a) DMSO/Ac₂O, rt, 16 h; (b) Ph₃P=CHCO₂Et, CHCl₃, 40 °C, 1.25 h, 33% overall yield; (c) Ph₃P=CHCO₂Et, CHCl₃, 40 °C, 1-2.5 h; 54–61%.



Scheme 3. Reagents and conditions: (a) BnN₃ or N₃CH₂(CH₂)₃CH=CH₂ (to give **26**), Cul/Amberlyst A-21 (cat.), CH₂Cl₂, rt, 16 h, 71% (**25**), 74% (**26**, along with **27**, 4%), 60% (**28**), 77% (**29**, along with **30**, 4%), 87% (**34**); (b) NaH, CH₂=CHCH₂Br, DMF, 50 °C, 1 h, 32% (**31**) and 20% (**32**); (c) DMSO/Ac₂O, rt, 16 h, 60%; (d) NEt₃/H₂O/MeOH, 40 °C, 16 h, 82–95% (**35**–**38**). Aglycons' carbon atoms numbering do not follow any nomenclature rules and were chosen to simplify the description of the NMR signals.

intermediate bis-copper complexes by the base [DMAP (4dimethylaminopyridine) or DMA (*N*,*N*-dimethylaniline)], allowing a further intermolecular delivery of iodine, was proposed.³² Although such a mechanism remains to be clarified, in our case

the presence of the basic dimethylamino groups in Amberlyst A-21 seems to be important for the iodination.

This protocol proved to be convenient due to the easy separation of the catalyst from the reaction medium, simply by filtration, and to the fully compatibility with the presence of the acetate functionality, avoiding the addition of base and the use of an organo/aqueous solvent system. To date, only one example of 'click' chemistry on carbohydrate templates by means of heterogeneous catalysis was reported, in which Cu(I)-modified zeolites were applied.³³

Variations on the sugar backbone substitution were also carried for investigating their influence on the bioactivity. The replacement of the acetyl protecting groups of **26** by allyl functions was achieved directly, along with OH-2 and N-allylation, by treatment with sodium hydride and allyl bromide in DMF at 50 °C. The fully allylated derivative **31** was thus obtained in 32% overall yield, together with **32**, having a free OH-3 (20%).

The combination of a triazole ring with a conjugated carbonyl system was then undertaken. Thus, oxidation of **28** by the DMSO/Ac₂O method furnished triazole-containing enuloside **33** in reasonable yield (60%). Dienepyranoside **22** was coupled through the alkynyl residue with benzyl azide using Cul/Amberlyst A-21 as catalyst at room temperature to generate the triazole derivative **34** in 87% yield. No competitive cycloaddition at the conjugated system was observed, even though such reactions between azides and α , β -unsaturated esters or conjugated dienes are known to occur, leading to triazolines³⁴ or aziridines.³⁵

Deprotection of the triazole-containing glycosides **25**, **26**, **28**, and **29** with triethylamine in methanol and water gave **35–38** (82–95%), a series of compounds bearing a hydrophilic part and a benzyl or a hexenyl moiety as a hydrophobic substituent at position 4 of the triazole ring.

2.2. Biological evaluation

2.2.1. Antimicrobial activity

The antimicrobial activities of 3-enopyranosid-2-uloses 13-15 and 18. dienepyranosides 21-24 and triazole derivatives 25-26. 28-29, 31-38 were investigated using the paper disk diffusion method.³⁶ Their in vitro antibacterial activity was evaluated against Gram-negative strains such as Escherichia coli and Salmonella enteritidis, and the following Gram-positive bacteria: Enterococcus faecalis, Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, and Bacillus subtilis. The antifungal activity was studied on a panel of plant pathogenic fungi which may also cause human allergies including Aspergillus niger, Aspergillus brasiliensis, Botrytis cinerea and Fusarium solani, the fungal plant pathogens Penicillium aurantiogriseum, and Fusarium culmorum and the human pathogen Candida albicans. The significant results are presented in Table 2. Given the variability of the method, the results are expressed by the average diameter of the inhibition zone detected in two replicates, as well as by the symbols -, +, ++, +++, ++++, +++++, corresponding to a range of diameters, for increasing sensitivity of the microorganism to the substance tested according to Miyazawa et al.³⁷ Chloramphenicol was used as control for all bacteria tested, whereas for fungi, actidione and amphotericin B were used.

Despite the presence of a triazole nucleus, which is known to play a vital role in the efficacy of many bioactive agents, compounds **25–26**, **28–29**, **31–38** did not show any significant antimicrobial activity in this screening. Pyranoid derivatives bearing conjugated carbonyl systems were found to be the relevant bioactive compounds of this set. From the 3-enopyranosid-2-uloses assayed, only those carrying a dodecyl chain were active (**15** and **18**) and their efficacy proved to be dependent on their anomeric configuration. The α -enuloside **15** displayed strong activity against *E. faecalis*, while the β -anomer **18** showed virtually no activity at all. Moreover **15** showed a very strong effect against the two species of *Bacillus* tested, with zones of inhibition presenting a diameter similar or greater than that of the standard antibiotic. Moderate and good activities were displayed by **18** against *B. cereus* and *B. subtilis*, respectively. The latter compound was more effective than its α -counterpart toward *S. enteritidis*, showing moderate activity, and toward the fungal pathogen *A. niger*, for which a very strong inhibitory effect was observed. In addition, both enones exhibited similar activity against *L. monocytogenes* (moderate effect) and *P. aurantiogriseum*. In the latter case the activities were found to be greater than those of the standard antibiotics.

Dienepyranosides **22–24** were shown to selectively inhibit the growth of bacteria, namely a strong activity against *E. faecalis* was detected. Compound **24** was the most active one toward *S. aureus*, displaying good effect.

Although no complete correlation between bioactivity and structure was found in these series of compounds, one obvious effect is that the bioactivity observed for the 3-enopyran-2-uloses depends strongly on the aglycon moiety. Since enones 13 and 14, having propargyl and benzyl moieties, did not exhibit any effect, the long and straight chain in 15 and 18 must play a role in eliciting the activity. The hydrophobic portion may allow these molecules to interact with the lipid bilayer of the microorganism cell membrane, or to pass through it. Once inside the lipid membrane or in the cytoplasm, the compound may act by binding to specific targets, especially enzymes involved in key biochemical pathways for microorganism growth. Such inhibition might arise from Michael type addition of enzymes' nucleophilic groups to the enone system, despite the presence of the electrondonating group at C-3. Concerning dienepyranosides 22-24, the hydrophobic nature of the N-substituent does not seem to be a major contributor for the observed bioactivity, with the exception of the effect on S. aureus, a microbe only susceptible to compound 24.

2.2.2. Acute toxicity

In the search for new therapeutic agents, one of the main prerequisites is that the new bioactive molecules should be toxic to the pathogen and exhibit minimal toxicities to the host cells. Hence, our further interest was to evaluate the potential toxicity of the new series of compounds, particularly that of the most bioactive ones. The in vitro acute toxicity in eukaryotic cells of all molecules submitted to antimicrobial evaluation was assessed using the MTT cell viability assay.³⁸ The results quantified as IC₅₀ values are summarized in Table 3. Most of the compounds exhibited low toxicity; the higher toxic effect was observed for the β -enuloside **18** with an IC₅₀ value of 0.045 mg/mL, while the less toxic molecule appeared to be the triazole-containing dienepyranoside 34 with an IC₅₀ value of 12 mg/mL. Among the molecules that displayed significant antimicrobial effects, 18 and 24 (IC₅₀ value of 0.076 mg/ mL) showed the highest toxicity. The α -enuloside **15** and dienepyranosides 22 and 23 showed low toxic effect, with IC₅₀ values of the same order of magnitude as that of the negative control (DMSO).

3. Conclusions

Highly functionalized sugar derivatives, structurally suitable for derivatization and for bioactivity screening, namely 3-enopyranosid-2-uloses and 2-C-branched-chain dienepyranosides, were straightforwardly accessed starting from CMGLs. Tri-O-acylated 2-hydroxy pyranosides, arising from the opening of the bicyclic lactones, were directly converted to the target enones by oxidation/elimination. The anomeric configuration influences the conformational stability of such enulosides and thus the yields obtained for their preparation. A further Wittig-type olefination

Table 2 Antimicrobial activities of the newly synthesized 3-enopyran-2-uloses 15, 18, and dienepyranosides 22–24

Compound		15		18		22		23		24	Со	ntrol ^{a,b}	Co	ontrol ^c
	Ø (mm)	inhibition	Ø (mm)	inhibition										
Bacteria ^d														
Salmonella enteritidis ATCC 13076	<12	-	14	++	<12	-	<12	-	<12	-	41	+++++		
Enterococcus faecalis ATCC	24	++++	<12	_	24	++++	24	++++	22	++++	37	+++++		
Listeria monocytogenes	17	++	14	++	<12	_	<12	_	<12	_	33	+++++		
Staphylococcus aureus ATCC 6538	17	++	13	+	<12	-	<12	-	20	+++	36	+++++		
Bacillus cereus ATCC 11778	39	+++++	16	++	<12	-	<12	-	<12	-	31	+++++		
Bacillus subtilis ATCC 6633	40	+++++	21	+++	<12	-	<12	-	12	+	40	+++++		
Fungi ^d														
Aspergillus niger ATCC 16404	12	+	40	+++++	<12	_	<12	_	<12	_	43	+++++	22	++++
Penicillium aurantiogriseum ATCC 16025	30	+++++	33	+++++	<12	_	12	+	12	+	26	+++++	26	+++++

Diameter of inhibition zones (Ø): +++++, Ø \ge 26 mm; ++++, 22 mm \le Ø < 26 mm; +++, 18 mm \le Ø < 22 mm; ++, 14 mm \le Ø < 18 mm; +, 12 mm \le Ø < 14 mm; -, Ø < 12 mm.

^a Chloramphenicol for all bacteria tested with the exception of fungi for which ^bactidione and ^camphotericine B was used.

^d Microorganisms collection of Microbiology Laboratory from Escola Superior Agrária–Instituto Politécnico de Santarém.

Table 3

IC₅₀ values of in vitro acute toxicity of 3-enopyran-2-uloses **13–15** and **18**, dienepyranosides **21–24** and triazole derivatives **25–26**, **28**, **29**, **32–38** in eukaryotic cells using the MTT cell viability assay

	IC ₅₀ (mg/mL)	SD
DMSO	0.199	0.037
H_2O_2	0.002	0.002
13	0.282	0.021
14	0.163	0.012
15	0.136	0.010
18	0.045	0.003
21	0.352	0.026
22	0.257	0.019
23	0.128	0.010
24	0.076	0.006
25	0.879	0.066
26	0.063	0.005
28	0.069	0.005
29	0.505	0.038
32	1.186	0.089
33	10.313	0.773
34	11.987	0.899
35	0.617	0.046
36	0.394	0.030
37	0.193	0.014
38	0.426	0.032

afforded dienepyranosides with (E)-configuration around the exocyclic double bond. Conversion of (N-propargylcarbamoyl)methyl glycosides into 1,2,3-triazole derivatives by cycloaddition with a terminal azide was accomplished in smooth condition using a heterogeneous Cul/Amberlyst catalytic system.

The antimicrobial evaluation demonstrated relevant activity for some of the synthesized enones and diene pyranosides. Only the most hydrophobic enones, that is, those containing dodecyl chains, were active and different antifungal and antibacterial response was observed for α - and β -anomers. Since the hydrophobicity appears to be essential for the bioactivity of the synthesized enones, is plausible to suppose that their mechanism of action is related to incorporation or penetration across the cell membrane. In both cases binding to specific receptors or enzymes may occur. Although the electrophilicity of the enone system is reduced by the presence of the acetoxy group at C-3, Michael acceptor ability of enones **15**, **18** could be considered to act as a second structural requirement for bioactivity expression, particularly if enzymatic inhibitory activity is involved. Among the dienepyranosides tested, the contribution for the activity of the N-substituent hydrophobicity was only observed for *S. aureus*, which was susceptible only to **24**.

The results of acute toxicity indicate that from the 19 newly synthesized compounds, only four (**18**, **24**, **26**, and **28**) can be considered toxic. Compounds **15**, **22**, and **23**, included among those which exhibited significant antimicrobial activities, showed low toxicity. This encourages further investigation on their use for the control of the pathogens for which efficacy was detected. Moreover, the low acute toxicity observed for most of the molecules screened, motivates the study of their effect towards other therapeutic targets.

4. Experimental section

4.1. Chemistry

4.1.1. General methods

All reactions were monitored by TLC on Macherei-Nagel 60 F_{254} silica gel aluminum plates with detection under UV light (254 nm) and/or by charring with a solution of 10% H_2 SO4 in EtOH. Column chromatography was carried out on Silica Gel 60 (0.040–0.063 mm, Macherey-Nagel). ¹H and ¹³C NMR spectra were acquired with a Bruker ALS 300, DRX300, (300 MHz for ¹H and 75 MHz for ¹³C) or DRX 400 (400 MHz for ¹H and 100 MHz for ¹³C) spectrometer. Chemical shifts are expressed in parts per million and are referenced to solvent residual peaks. Assignments were made by COSY,

HSQC, HMBC and, when necessary, by NOESY experiments. HRMS spectra were recorded by the Centre Commun de Spectrometrie de Masse, Université Claude Bernard Lyon 1 (Villeurbanne) using ESI technique. Optical rotations were measured on a Perkin–Elmer 241 polarimeter at 20 °C (589 nm, sodium D line). Melting points were determined with a Stuart Scientific SMP 3 apparatus and are uncorrected.

4.1.2. General procedure for the opening of the CMGLs with amines

To a solution of CMGL (0.150 g, 0.43 mmol) in anhydrous CH_2Cl_2 (2.5 mL) was added the amine (0.47 mmol, 1.1 equiv) and the reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. After concentration under vacuum, the residue was purified by column chromatography on silica gel.

4.1.2.1. (N-Benzylcarbamovl)methyl 3.4.6-tri-O-acetyl-α-p-gluco-Reaction of α -CMG lactone 1^{18a} (0.10 g, pyranoside (5). 0.29 mmol) with benzylamine according to general procedure gave the title compound (87 mg, 66%) as a colorless oil after purification by column chromatography (from EtOAc/pentane, 3:2 to EtOAc); $R_{\rm f} = 0.21$ (EtOAc/pentane, 1:1). $[\alpha]_{\rm D}^{20} = +60$ (c 0.8, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.88 (t, 1H, NH, J = 5.8), 7.27–7.12 (m, 5H, Ph), 5.18 (t, 1H, H-3, $J_{2,3} = J_{3,4} = 9.8$), 4.90 (t, 1H, H-4, $J_{3,4} = J_{4,5}$), 4.79 (d, 1H, H-1, J_{1,2} = 3.8), 4.33-4.23 (m, 3H, CH₂-9, OH), 4.18 (dd, part A of ABX system, H-6a, $J_{5,6a}$ = 4.5, $J_{6a,6b}$ = 12.3), 4.10-3.87 (m, 4H, H-5, H-6b, CH_2 -7, $J_{7a,7b}$ = 15.6), 3.70–3.61 (m, 1H, H-2), 1.99 (s, 3H, Me, Ac), 1.94 (s, 6H, $2 \times$ Me, Ac); ¹³C NMR (100 MHz, CDCl₃) δ 171.7, 170.7, 169.6, 169.3 (3 × CO-Ac, CO-8), 137.9 (Cq, Ph), 128.6, 127.5, 127.4 (CH, Ph), 99.3 (C-1), 73.3 (C-3), 70.2 (C-2), 68.1, 68.0, 67.4 (C-4, C-5, C-7), 61.9 (C-6), 42.8 (C-9), 20.8, 20.7, 20.6 (3 \times Me, Ac); HRMS: calcd for C₂₁H₂₇NO₁₀ [*M*+Na]⁺ 476.1533, found 476.1535.

4.1.2.2. (N-Propargylcarbamoyl)methyl 3,4,6-tri-O-acetyl-β-D-Reaction of β -CMG lactone **3**^{18c} glucopyranoside (8). (0.11 g, 0.32 mmol) with *N*-propargylamine according to general procedure gave the title compound (0.11, 86%) as a pale oil after purification by column chromatography (from EtOAc/pentane, 3:2 to 4:1). $R_{\rm f} = 0.35$ (EtOAc/pentane, 4:1); $[\alpha]_{\rm D}^{20} = -3$ (c 0.4, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 5.09–5.01 (m, 2H, H-3, H-4), 4.41 (d, 1H, H-1, J_{1,2} = 7.9), 4.37–4.21 (m, 3H, H-6a, CH₂-7, $J_{5,6a} = 5.1$, $J_{6a,6b} = 12.4$, $J_{7a,7b} = 16.1$), 4.15-4.06 (m, 3H, H-6b, CH_2 -9), 3.74–3.62 (m, 2H, H-2, H-5), 2.23 (t, 1H, H-11, J = 2.6), 2.11 (s, 3H, Me, Ac), 2.09 (s, 3H, Me, Ac), 2.04 (s, 3H, Me, Ac); ¹³C NMR (100 MHz, CDCl₃) δ 171.7, 170.8, 169.7, 169.0 (3 × CO-Ac, CO-8), 103.4 (C-1), 79.2 (C-10),75.6 (C-3), 72.4, 72.3 (C-2, C-5), 71.8 (C-11), 69.4 (C-7), 67.9 (C-4), 62.0 (C-6), 28.8 (C-9), 21.0, 20.9, 20.8 $(3 \times Me, Ac)$; HRMS: calcd for $C_{17}H_{23}NO_{10}$ [*M*+Na]⁺ 424.1220, found 424.1219.

4.1.2.3. (N-Benzylcarbamoyl)methyl 3,4,6-tri-O-acetyl-β-D-gluco-Reaction of β -CMG lactone **3**^{18c} (0.15 g, pyranoside (9). 0.43 mmol) with N-benzylamine according to general procedure gave the title compound (0.185 g, 94%) as a colorless oil after purification by column chromatography (EtOAc/pentane, 3:2 to 4:1). $R_{\rm f}$ = 0.38 (EtOAc/pentane, 4:1); $[\alpha]_{\rm D}^{20}$ = -6 (c 0.9, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.40 (t, 1H, NH), 7.29-7.15 (m, 5H, Ph), 5.06-4.93 (m, 2H, H-3, H-4, $J_{2,3} = J_{3,4} = J_{4,5} = 9.3$), 4.48 (dd, part A of ABX system, 1H, H-9a, *J*_{9a,NH} = 6.0, *J*_{a,b} = 14.9), 4.40–4.15 (m, 5H, H-1, H-9b, H-6a, CH₂-7, $J_{1,2}$ = 7.8, $J_{5,6a}$ = 4.8, $J_{6a,6b}$ = 12.6, $J_{7a,7b}$ = 15.9, $J_{9b,NH}$ = 5.8), 4.03 (dd, part B of ABX system, H-6b, $J_{5,6b}$ = 2.0), 3.64 (ddd, 1H, H-5), 3.57 (dd, 1H, H-2), 2.04 (s, 3H, Me, Ac), 2.03 (s, 3H, Me, Ac), 2.01 (s, 3H, Me, Ac); ^{13}C NMR (100 MHz, CDCl₃) δ 171.2, 170.7, 169.7, 169.3 (3 × CO-Ac, CO-8), 137.9 (Cq, Ph), 128.8, 127.7, 127.6 (CH, Ph), 103.4 (C-1), 75.2 (C-3), 72.1, 72.0 (C-2, C-5), 69.2

(C-7), 68.1 (C-4), 61.9 (C-6), 43.0 (C-9), 20.9, 20.8, 20.7 (3 \times Me, Ac); HRMS: calcd for $C_{21}H_{27}NO_{10}~\left[\textit{M}+Na\right]^{*}$ 476.1533, found 476.1533.

4.1.2.4. (*N*-Dodecylcarbamoyl)methyl 3,4,6-tri-O-acetyl-β-D-Reaction of β -CMG-2-0-lactone **3**^{18c} glucopyranoside (10). (0.107 g, 0.31 mmol) with N-dodecylamine according to general procedure gave the title compound (0.146 g, 89%) as a colorless oil after purification by column chromatography (EtOAc/pentane, 3:2). $R_{\rm f}$ = 0.31 (EtOAc/pentane, 3:2); $[\alpha]_{\rm D}^{20}$ = -11 (*c* 0.7, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.04 (t, 1H, NH, J = 5.7), 5.08–4.97 (m, 2H, H-3, H-4), 4.38 (d, 1H, H-1, J_{1,2} = 7.9), 4.31–4.22 (m, 2H, H-6a, H-7a, $J_{5,6a} = 5.1$, $J_{6a,6b} = 12.4$, $J_{7a,7b} = 15.8$), 4.16 (d, part B of AB system, 1H, H-7b), 4.07 (dd, part B of ABX system, 1H, H-6b, J_{5.6b} = 2.3), 3.93 (d, 1H, OH), 3.73–3.57 (m, 2H, H-2, H-5), 3.31– 3.14 (m, 2H, CH₂-9), 2.07 (s, 3H, Me, Ac), 2.06 (s, 3H, Me, Ac), 2.02 (s, 3H, Me, Ac), 1.54-1.41 (m, 2H, CH₂-10), 1.32-1.19 (m, 18 H, C_9H_{18}), 0.86 (t, 3H, CH_3 -20, J = 6.6); ¹³C NMR (100 MHz, $CDCl_3$) δ 171.4, 170.7, 169.7, 169.2 (3 × CO-Ac, CO-8), 103.3 (C-1), 75.5 (C-3), 72.2, 72.2 (C-2, C-5), 69.1 (C-7), 68.0 (C-4), 62.0 (C-6), 39.3 (C-9), 32.0, 29.7, 29.7, 29.5, 29.4, 29.4, 27.0, 22.8 (C-10-C-19), 20.9, 20.8, 20.7 (2 \times Me, Ac), 14.2 (CH₃-20); HRMS: calcd for C₂₆H₄₅NO₁₀ [*M*+Na]⁺ 554.2941, found 554.2940.

4.1.3. General procedure for PDC/Ac₂O oxidation of 4, 7

A solution of 3,4,6-tri-O-acetylglycopyranoside (0.1 g, 0.25 mmol) in dry CH_2Cl_2 (0.7 mL) was added to a mixture of PDC (70 mg, 0.19 mmol) and Ac_2O (0.07 mL, 0.8 mmol) in dry CH_2Cl_2 (1.5 mL) under nitrogen atmosphere The resulting mixture was stirred under reflux for 1 h, then cooled to rt. The solvent was removed in vacuo. The gummy residue was triturated with ethyl acetate (15 mL) and the mixture was filtered through a short pad of Florisil. After evaporation of the solvent, the crude was purified by column chromatography (EtOAc/pentane, 3:2).

4.1.4. General procedure for Dess-Martin oxidation of 4, 7

To a solution of 3,4,6-tri-O-acetylglycopyranoside (0.3 mmol) in dry CH_2Cl_2 (6 mL) was added Dess–Martin periodinane (0.165 g, 0.39 mmol), and the reaction mixture was stirred overnight at room temperature under nitrogen atmosphere. A saturated NaH- CO_3 solution was added, and the aqueous phase was extracted twice with dichloromethane. The combined organic layers were washed with water and dried with Na₂SO₄. After filtration and evaporation of the solvent, the residue was purified by column chromatography (EtOAc/pentane, 3:2).

4.1.4.1. (N-Propargylcarbamoyl)methyl 3,4,6-tri-O-acetyl-α-D-Oxidation of **4**^{18a} with arabino-hexopyranosid-2-ulose (11). PDC/Ac₂O (0.1 g, 0.25 mmol of **4**) or with Dess-Martin periodinane (0.12 g, 0.3 mmol of 4), according to general procedures, afforded 11 (15 mg and 18 mg, respectively, 15% yield in both cases), inseparably contaminated with compound **13**. $R_f = 0.22$ (EtOAc/pentane, 1:1); ¹H NMR (300 MHz, CDCl₃) δ 5.53 (d, 1H, H-3, $J_{3,4}$ = 9.6), 5.20 (t, 1H, H-4, J_{3,4 ~} J_{4,5}), 5.38, 4.91 (s, 1H, H-1), 4.54 -4.49 (d, part A of AB system, H-7a, $J_{7a,7b}$ = 16.9), 4.38 (d, part B of AB system part, H-7b), 4.34-4.25 (m, 3H, H-5, H-6a, H-9a) 4.16-4.10 (m, 2H, H-6b, H-9b), 2.15 (t, 1H, H-11, J = 2.5), 2.12 (s, 3H, Me, Ac), 2.08 (m, 3H, Me, Ac), 2.08 (s, 3H, Me, Ac); 13 C NMR (75 MHz, CDCl₃) δ 170.9, 170.1, 169.9, 165.7 (2 × CO-Ac, CO-8), 97.5 (C-1), 79.5 (C-10), 75.0 (C-3), 70.7 (C-5), 70.4 (C-11), 67.6 (C-4), 66.8 (C-7), 62.2 (C-6), 30.5 (C-9), 21.5, 20.9, 20.8 (3 \times Me, Ac); LRMS (ESI) m/ $z = 422.1 [M+Na]^+, 438.0 [M+K]^+, 820.6 [M_2Na]^+.$

4.1.4.2. (*N*-**Propargylcarbamoyl**)**methyl 3,4,6-tri-O-acetyl-\alpha-D***lyxo***-hexopyranosid-2-ulose** (**12**). Oxidation of **7**^{18b} with PDC/Ac₂O or with Dess–Martin periodinane according to general procedures, afforded **12** in 5% yield (5 mg, starting from 0.1 g of **7**), and in 22% yield (23 mg, starting from 0.105 g of **10**), respectively, inseparably contaminated with compound **13**. $R_f = 0.22$ (EtOAc/ pentane, 1:1); ¹H NMR (300 MHz, CDCl₃) δ 5.50 (dd, 1H, H-4, $J_{3,4} = 3.5$, $J_{4,5} = 1.5$), 5.38 (d, 1H, H-3), 4.91 (s, 1H, H-1), 4.56–4.49 (m, 2H, H-5, H-7a, $J_{7a,7b} = 16.9$), 4.38 (d, part B of AB system part, H-7b), 4.29 (dd, part A of ABX system, 1H, H-9a, $J_{9a,H-11} = 2.0$, $J_{9a,9b} = 16.9$), 4.26–4.09 (m, 3H, CH_2 –6, H-9b, $J_{5,6a} = 6.0$, $J_{5,6b} = 7.1$, $J_{6a,6b} = 11.6$), 2.21 (s, 3H, Me, Ac), 2.14–2.10 (m, 4H, H-11,Me, Ac), 2.06 (s, 3H, Me, Ac); ¹³C NMR (75 MHz, CDCl₃) δ 170.5, 169.5, 169.1, 165.3 (2 × CO-Ac, CO-8), 98.7 (C-1), 80.7 (C-10), 71.3 (C-3), 70.2 (C-11), 68.6 (C-5), 67.5 (C-4), 66.8 (C-7), 61.3 (C-6), 30.8 (C-9), 21.3, 20.8, 20.8 (3 × Me, Ac); HRMS: calcd for C₁₇H₂₁NO₁₀ [*M*+Na]⁺ 422.1063, found 422.1059.

4.1.5. General procedure for DMSO/Ac₂O oxidation

To a solution of 3,4,6-tri-O-acetylglycopyranoside (0.38 mmol) in anhydrous DMSO (25 mL) was added acetic anhydride (12.5 mL). The mixture was stirred at room temperature overnight under nitrogen atmosphere. Water (50 mL) was added and the resulting mixture was extracted with EtOAc (3×25 mL). The combined organic layers were washed with water and brine and dried with Na₂SO₄. After filtration, the solvent was removed under vacuum and the crude was purified by column chromatography on silica gel.

4.1.5.1. (N-Propargylcarbamoyl)methyl 3,6-di-O-acetyl-4-deoxy- α -D-glycero-hex-3-enopyranosid-2-ulose (13). DMSO/Ac₂O oxidation of compound **4**^{18a} (0. 123 g, 0.31 mmol) or **7** (0. 15 g, 0.37 mmol) according to general procedure gave the corresponding 3-enopyranosid-2-ulose 13 (63 mg, 61% and 74 mg, 59%, respectively) as a colorless oil after purification by column chromatography (EtOAc/pentane, 1:1). $R_{\rm f} = 0.31$ (EtOAc/pentane, 1:1); $[\alpha]_{\rm p}^{20} = +7$ (c 0.9, CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 6.77 (t, 1H, NH), 6.65 (d, 1H, H-4, J_{4,5} = 1.9), 5.07 (s, 1H, H-1), 4.95 (td, 1H, H-5), 4.42 (dd, part A of ABX system, 1H, H-6a, J_{5,6a} = 5.3, J_{6a,6b} = 11.7), 4.31 (d, part A of AB system, 1H, H-7a, $J_{7a,7b}$ = 15.3), 4.22 (dd, part B of ABX system, 1H, H-6b, $J_{5.6b}$ = 4.5), 4.16 (d, part B of AB system, 1H, H-7b), 4.09-4.02 (m, 2H, CH₂-9), 2.24 (s, 3H, Me, Ac), 2.23 (t, 1H, H-11, I = 2.6), 2.08 (s, 3H, Me, Ac); ¹³C NMR (75 MHz, CDCl₃) δ 181.7 (CO-2), 170.7, 168.0, 167.8 (2 × CO-Ac, CO-8), 141.9 (C-3), 133.3 (C-4), 98.1 (C-1), 79.1 (C-10), 71.8 (C-11), 68.4, 68.3 (C-5, C-7), 64.3 (C-6), 28.8 (C-9), 20.8, 20.4 (2 × Me, Ac); HRMS: calcd for C₁₅H₁₇NO₈ [*M*+Na]⁺ 362.0852, found 362.0851.

4.1.5.2. (N-Benzylcarbamoyl)methyl 3,6-di-O-acetyl-4-deoxy-αp-glycero-hex-3-enopyranosid-2-ulose (14). DMSO/Ac₂O oxidation of compound 5 (80 mg, 0.18 mmol) according to general procedure gave the corresponding 3-enopyranosid-2-ulose 14 (66 mg, 96%) as a colorless oil after purification by column chromatography (EtOAc/pentane, 3:2). $R_f = 0.31$ (EtOAc/pentane, 1:1); $[\alpha]_{D}^{20}$ = +13 (c 0.7, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.36–7.24 (m, 5H, Ph), 6.86 (br t, 1H, NH), 6.62 (d, 1H, H-4, J_{4.5} = 1.9), 5.04 (s, 1H, H-1), 4.93 (td, 1H, H-5), 4.51-4.32 (m, 4H, H-6a, H-7a, CH₂-9, $J_{5,6a} = 5.3$, $J_{6a,6b} = 11.9$, $J_{7a,7b} = 15.6$), 4.26–4.18 (m, 2H, H-6b, H-7b, $J_{5,6b}$ = 4.5), 2.24 (s, 3H, Me, Ac), 2.10 (s, 3H, Me, Ac); ¹³C NMR (100 MHz, CDCl_3) δ 181.8 (CO-2), 170.7, 168.0, 167.9 (2 \times CO-Ac, CO-8), 141.9 (C-3), 137.9 (Cq, Ph), 133.2 (C-4), 128.8, 127.9, 127.7 (CH, Ph), 98.2 (C-1), 68.3 (C-5, C-7), 64.4 (C-6), 99.3 (C-1), 73.3 (C-3), 70.2 (C-2), 68.4 (C-5, C-7), 64.3 (C-6), 43.1 (C-9), 20.8, 20.4 $(2 \times Me, Ac)$; HRMS: calcd for C₁₉H₂₁NO₈ [*M*+Na]⁺ 414.1165, found 414.1167.

4.1.5.3. (*N*-Dodecylcarbamoyl)methyl **3,6-di-O-acetyl-4-deoxy**- α -*p-glycero-hex-3-enopyranosid-2-ulose* (**15**). DMSO/Ac₂O oxidation of compound **6**^{18a} (0.12 g, 0.23 mmol) according to general procedure gave the corresponding 3-enopyranosid-2-ulose **15**

(0.105 g, 99%) as a yellow oil after purification by column chromatography (EtOAc/pentane, 1:1). $R_{\rm f}$ = 0.25 (EtOAc/pentane, 2:3); $[\alpha]_{20}^{20}$ = +30 (*c* 1, CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 6.65 (d, 1H, H-4, $J_{4,5}$ = 1.9), 6.49 (t, 1H, NH), 5.02 (s, 1H, H-1), 4.95 (td, 1H, H-5), 4.42 (dd, part A of ABX system, 1H, H-6a, $J_{5,6a}$ = 5.5, $J_{6a,6b}$ = 11.8), 4.31–4.21 (m, 2H, H-6b, H-7a, $J_{5,6b}$ = 4.5, $J_{7a,7b}$ = 15.1), 4.15 (d, part B of AB system, 1H, H-7b), 3.33–3.18 (m, 2H, CH₂-9), 2.26 (s, 3H, Me, Ac), 2.10 (s, 3H, Me, Ac), 1.55–1.45 (m, 2H, CH₂-10), 1.33–1.20 (m, 18 H, C₉H₁₈), 0.86 (t, 3H, CH₃-20, *J* = 7.1); ¹³C NMR (75 MHz, CDCl₃) δ 181.9 (CO-2), 170.7, 167.9, 167.8 (2 × CO-Ac, CO-8), 142.0 (C-3), 133.2 (C-4), 98.2 (C-1), 68.3 (C-5, C-7), 64.4 (C-6), 39.3 (C-9), 32.0, 29.8, 29.7, 29.6, 29.5 29.4, 29.4, 27.0, 22.8 (C-10–C19), 20.8, 20.4 (2 × Me, Ac), 14.2 (CH₃-20); HRMS: calcd for C₂₄H₃₉NO₈ [*M*+Na]⁺ 492.2573, found 492.2574.

4.1.5.4. (*N*-Propargylcarbamoyl)methyl **3,6-di-***O*-acetyl-**4-deoxy**β-D-*glycero*-hex-**3-enopyranosid-2-ulose** (**16**). DMSO/Ac₂O oxidation of compound **8** (0.1 g, 0.25 mmol) according to general procedure gave the corresponding 3-enopyranosid-2-ulose **16** (27 mg, 32%) as a colorless oil after purification by column chromatography (EtOAc/pentane, 3:2). $R_{\rm f}$ = 0.48 (EtOAc/pentane, 4:1); ¹H NMR (300 MHz, CDCl₃) δ 7.00 (t, 1H, NH), 6.70 (d, 1H, H-4, $J_{4,5}$ = 3.0), 5.07 (s, 1H, H-1), 4.95–4.89 (ddd, 1H, H-5), 4.47–4.25 (m, 3H, H-6a, H-6b, H-7a, $J_{5,6a}$ = 6.6, $J_{5,6b}$ = 4.9, $J_{6a,6b}$ = 11.7, $J_{7a,7b}$ = 15.7), 4.21 (d, part B of AB system, 1H, H-7b), 4.12–4.06 (m, 2H, CH₂-9), 2.27 (s, 3H, Me, Ac), 2.23 (t, 1H, H-11, J = 2.6), 2.12 (s, 3H, Me, Ac); HRMS: calcd for C₁₅H₁₇NO₈ [*M*+Na]⁺ 362.0852, found 362.0852.

4.1.5.5. (*N*-Benzylcarbamoyl)methyl 3,6-di-O-acetyl-4-deoxy-β *p-glycero*-hex-3-enopyranosid-2-ulose (17). DMSO/Ac₂O oxidation of compound **9** (0.11 g, 0.24 mmol) according to general procedure gave the corresponding 3-enopyranosid-2-ulose **17** (42 mg, 45%) as a colorless oil after purification by column chromatography (from EtOAc/pentane, 3:2 to 4:1); $R_{\rm f}$ = 0.49 (EtOAc/pentane, 4:1); ¹H NMR (400 MHz, CDCl₃) δ 7.36–7.24 (m, 5H, Ph), 7.13 (br t, 1H, NH), 6.67 (d, 1H, H-4, $J_{4,5}$ = 3.0), 5.05 (s, 1H, H-1), 4.89 (ddd, 1H, H-5), 4.53–4.33 (m, 4H, H-6a, H-7a, CH₂-9, $J_{7a,7b}$ = 15.4, $J_{9,NH}$ = 5.8, $J_{9a,9b}$ = 9.2), 4.32–4.18 (m, 2H, H-6b, H-7b, $J_{5,6b}$ = 5.1, $J_{6a,6b}$ = 11.7), 2.24 (s, 3H, Me, Ac), 2.08 (s, 3H, Me, Ac); HRMS: calcd for C₁₉H₂₁NO₈ [*M*+Na]⁺ 414.1165, found 414.1165.

4.1.5.6. (N-Dodecylcarbamoyl)methyl 3,6-di-O-acetyl-4-deoxy- β -D-glycero-hex-3-enopyranosid-2-ulose (18). $DMSO/Ac_2O$ oxidation of compound 10 (0.1 g, 0.19 mmol) according to general procedure gave the corresponding 3-enopyranosid-2-ulose 18 (33 mg, 37%) as a colorless oil after purification by column chromatography (from EtOAc/pentane, 3:2 to 4:1). $R_f = 0.43$ (EtOAc/pentane, 3:2); ¹H NMR (300 MHz, CDCl₃) δ 6.74 (t, 1H, NH), 6.69 (d, 1H, H-4, J_{4,5} = 3.2), 5.03 (s, 1H, H-1), 4.94–4.87 (m, 1H, H-5), 4.43–4.26 (m, 3H, H-6a, H-6b, H-7a, $J_{5,6a} = 6.8$, $J_{5,6b} = 5.1$, $J_{6a,6b} = 11.5$, $J_{7a,7b} = 15.5$), 3.31 - 3.22 (m, 2H, CH₂-9), 2.26 (s, 3H, Me, Ac), 2.11 (s, 3H, Me, Ac), 1.57-1.44 (m, 2H, CH2-10), 1.33-1.21 (m, 18 H, C_9H_{18}), 0.87 (t, 3H, CH_3 -20, J = 6.8); ¹³C NMR (75 MHz, CDCl₃) δ 182.5 (CO-2), 170.6, 168.3, 168.0 (2 × CO-Ac, CO-8), 142.4 (C-3), 133.1 (C-4), 99.1 (C-1), 71.2, 68.7 (C-5, C-7), 65.2 (C-6), 39.3 (C-9), 32.0, 29.8, 29.7, 29.6, 29.5, 29.4, 27.0, 22.8 (C-10-C19), 20.8, 20.4 (2 × Me, Ac), 14.2 (CH₃-20); HRMS; calcd for C₂₄H₃₉NO₈ [*M*+Na]⁺ 492.2573, found 492.2574.

4.1.6. General procedure for Wittig olefination of 3-enopyranosid-2-uloses

To a solution of 3-enopyranosid-2-ulose (0.10 mmol) in $CHCl_3$ (1 mL) was added [(ethoxycarbonyl)methylene]triphenylphosphorane (45 mg, 0.13 mmol). The whole solution was stirred at 40 °C

until complete conversion, as indicated by TLC. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel.

4.1.6.1. (*N*-Allylcarbamoyl)methyl 3,6-di-O-acetyl-4-deoxy- α -D-glycero-hex-3-enopyranosid-2-ulose (20) and (*N*-Allylcarbamoyl)methyl 3,6-di-O-acetyl-2,4-dideoxy-2-C-[(*E*)-(ethoxycarbonyl)methylene]- α -D-glycero-hex-3-enopyranoside

(21). DMSO/Ac₂O oxidation of compound 19^{18c} (75 mg, 0.19 mmol) to 3-enopyranosid-2-ulose **20** was performed according to general procedure. After column chromatography (EtOAc/pentane, 3:2), **20** was subjected to Wittig olefination which was completed within 1.25 h. Purification by column chromatography (from Et₂O/hexane, 9:1 to Et₂O) afforded **21** (25 mg, 33% overall yield), as white solid.

Data for **20**: $R_f = 0.42$ (EtOAc/pentane, 3:2); ¹H NMR (300 MHz, CDCl₃) δ 6.65 (d, 1H, H-4, $J_{4,5} = 1.9$), 6.61 (br t, 1H, NH), 5.91–5.74 (m, 1H, H-10), 5.24–5.01 (m, 2H, CH_2 –11), 5.04 (s, 1H, H-1), 4.96 (td, 1H, H-5), 4.42 (dd, part A of ABX system, 1H, H-6a, $J_{5,6a} = 5.3$, $J_{6a,6b} = 11.7$), 4.32 (d, part A of AB system, 1H, H-7a, $J_{7a,7b} = 15.3$), 4.23 (dd, part B of ABX system, 1H, H-6b, $J_{5,6a} = 4.7$), 4.18 (d, part B of AB system 1H, H-7b) 3.95–3.87 (m, 2H, CH_2 –9), 2.25 (s, 3H, Me, Ac), 2.10 (s, 3H, Me, Ac); ¹³C NMR (75 MHz, CDCl₃) δ 181.8 (CO-2), 170.7, 167.9, 167.9 (2 × CO-Ac, CO-8), 141.9 (C-3), 133.3, 133.5 (C-4, C-10), 116.8 (C-11) 98.2 (C-1), 68.3, 68.3 (C-5, C-7), 64.3 (C-6), 41.5 (C-9), 20.8, 20.4 (2 × Me, Ac).

Data for **21**: $R_f = 0.24$ (EtOAc/pentane, 2:3); mp 101–103 °C; $[\alpha]_D^{20} = +50$ (*c* 0.7, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.11 (t, 1H, NH), 6.39 (s, 1H, H-1), 5.95 (dd, 1H, H-4, $J_{2',4} = 0.8$, $J_{4,5} = 2.0$), 5.91 (br d, 1H, H-2'), 5.89–5.80 (m, 1H, H-10), 5.19 (dq, 1H, H-11a, $J_{11a,11b} = J_{9a,11a} = J_{9b,11a} = 1.5$, $J_{10,11a} = 17.1$), 5.11 (dq, 1H, H-11b, $J_{9a,11b} = J_{9b,11b} = J_{11a,11b}$, $J_{10,11b} = 10.3$), 4.72 (ddd, 1H, H-11b, $J_{9a,11b} = J_{9b,11b} = J_{11a,11b}$, $J_{10,11b} = 10.3$), 4.72 (ddd, 1H, H-5), 4.40–4.30 (m, 2H, H-6a, H-7a, $J_{5,6a} = 5.8$, $J_{6a,6b} = 11.8$, $J_{7a,7b} = 15.6$), 4.27 (d, part B of AB system, 1H, H-7b), 4.23–4.15 (m, 2H, H-6b, CH₂-Et, $J_{5,6a} = 4.3$, $J_{Et} = 7.1$), 3.97–3.91 (m, 2H, CH₂-9), 2.27 (s, 3H, Me, Ac), 2.10 (s, 3H, Me, Ac), 1.29 (t, 3H, CH₃-Et); ¹³C NMR (100 MHz, CDCl₃) δ 170.9, 168.9, 168.0 (2 × CO-Ac, CO-8), 165.4 (CO), 142.1, 140.3 (C-2, C-3), 134.2 (C-10), 121.4 (C-4), 116.4 (C-11), 114.3 (C-2'), 94.2 (C-1), 67.1, 66.7 (C-5, C-7), 64.9 (C-6), 61.2 (CH₂-Et), 41.5 (C-9), 21.0, 20.9 (2 × Me, Ac), 14.3 (CH₃-Et); HRMS: calcd for C₁₉H₂₅NO₉ [*M*+Na]⁺ 434.1427, found 434.1426.

4.1.6.2. (N-Propargylcarbamoyl)methyl 3,6-di-O-acetyl-2,4dideoxy-2-C-[(E)-(ethoxycarbonyl)methylene]-a-p-glycero-hex-3-enopyranoside (22). Wittig olefination of the 3-enopyranosid-2-ulose 13 (28 mg, 0.08 mmol) according to general procedure, was completed within 2.5 h. The title compound was obtained as a white solid (19 mg, 56%) after purification by column chromatography (diethyl ether/hexane, 9:1 to diethyl ether). $R_{\rm f}$ = 0.30 (EtOAc/pentane, 2:3); mp 106–108 °C; $[\alpha]_{\rm D}^{20}$ = +31 (c 0.4, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.34 (t, 1H, NH), 6.38 (s, 1H, H-1), 5.95 (dd, 1H, H-4, *J*_{2',4} = 1.0, *J*_{4,5} = 2.0), 5.92 (br d, 1H, H-2'), 4.72 (ddd, 1H, H-5), 4.39–4.31 (m, 2H, H-6a, H-7a, J_{5.6a} = 5.8, $J_{6a,6b}$ = 11.8, $J_{7a,7b}$ = 15.6), 4.29–4.14 (m, 4H, H-6b, H-7b, CH₂-Et, $J_{5,6a}$ = 4.3, J_{Et} = 7.1), 4.13–4.06 (m, 2H, CH₂-9, $J_{H-9,H-11}$ = 2.5, $J_{H-9,H-11}$ _{9,NH} = 5.5), 2.27 (s, 3H, Me, Ac), 2.19 (t, 1H, H-11), 2.10 (s, 3H, Me, Ac), 1.31 (t, 1H, CH₃-Et); ¹³C NMR (100 MHz, CDCl₃) δ 170.9, 168.9, 168.0 (2 × CO-Ac, CO-8), 165.5 (CO), 142.1, 140.2 (C-2, C-3), 121.4 (C-4), 114.4 (C-2'), 94.3 (C-1), 79.6 (C-10), 71.3 (C-11), 67.1, 66.7 (C-5, C-7), 64.9 (C-6), 61.3 (CH₂-Et), 28.6 (C-9), 20.9, 20.9 (2 \times Me, Ac), 14.3 (CH₃-Et); HRMS: calcd for C₁₉H₂₃NO₉ [*M*+Na]⁺ 432.1271, found 432.1272.

4.1.6.3. (*N*-Benzylcarbamoyl)methyl **3,6-di-O-acetyl-2,4-dide-oxy-2-C-**[(*E*)-(ethoxycarbonyl)methylene]-α-D-glycero-hex-3-enopyranoside (23). Wittig olefination of the 3-enopyrano-

sid-2-ulose 14 (40 mg, 0.1 mmol) according to general procedure, was completed within 2 h. The title compound was obtained as a white solid (29 mg, 61%) after purification by column chromatography (diethyl ether/hexane, 7:3 to diethyl ether). $R_{\rm f} = 0.23$ (EtOAc/pentane, 2:3); mp 123–125 °C; $[\alpha]_D^{20}$ = +27 (c 0.3, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.52 (t, 1H, NH), 7.32–7.28 (m, 3H, Ph), 7.27-7.25 (m, 2H, Ph), 6.38 (s, 1H, H-1), 5.93 (dd, 1H, H-4, *J*_{2′.4} = 1.0, *J*_{4.5} = 2.3), 5.86 (br d, 1H, H-2′), 4.70 (ddd, 1H, H-5), 4.54 (dd, part A of ABX system, H-9a, $J_{9a,NH} = 6.0$, $J_{9a,9b} = 14.9$), 4.46 (dd, part B of ABX system, H-9b, $J_{9b,NH}$ = 5.8), 4.41–4.32 (m, 2H, H-6a, H-7a, $J_{5,6a}$ = 5.8, $J_{6a,6b}$ = 11.6, $J_{7a,7b}$ = 15.6), 4.29 (d, part B of AB system, H-7a), 4.16 (dd, part B of ABX system, H-6b, J_{5,6b} = 4.0), 4.10-3.97 (m, 2H, CH₂-Et), 2.24 (s, 3H, Me, Ac), 2.10 (s, 3H, Me, Ac), 1.21 (t, 1H, CH_3 -Et, J = 7.1); ¹³C NMR (100 MHz, CDCl₃) δ 170.9, 169.0, 168.0 (2 × CO-Ac, CO-8), 165.4 (CO), 142.1, 140.3 (C-2, C-3), 138.3 (Cq, Ph), 128.6, 128.0, 127.4 (CH, Ph), 121.4 (C-4), 114.3 (C-2'), 94.3 (C-1), 67.1, 66.6 (C-5, C-7), 64.9 (C-6), 61.2 (CH₂-Et), 43.1 (C-9), 20.9, 20.9 (2 × Me, Ac), 14.2 (CH₃-Et); HRMS: calcd for $C_{23}H_{27}NO_9$ [*M*+Na]⁺ 484.1584, found 484.1591.

4.1.6.4. (*N*-Dodecylcarbamoyl)methyl 3,6-di-O-acetyl-2,4-dideoxy-2-C-[(*E*)-(ethoxycarbonyl)methylene]-α-D-glycero-hex-3-

enopyranoside (24). Wittig olefination of the 3-enopyranosid-2-ulose 15 (40 mg, 0.09 mmol) according to general procedure, was completed within 1 h. The title compound was obtained as a white solid (25 mg, 54%) after purification by column chromatography (diethyl ether/hexane, 4:1 to diethyl ether). $R_{\rm f} = 0.43$ (EtOAc/pentane, 2:3); $[\alpha]_D^{20} = +26$ (c 0.5, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.04 (t, 1H, NH), 6.37 (s, 1H, H-1), 5.95 (dd, 1H, H-4, *J*_{2',4} = 0.8, *J*_{4,5} = 2.0), 5.91 (br d, 1H, H-2'), 4.71 (ddd, 1H, H-5), 4.37 (dd, part A of ABX system, H-6a, J_{5.6a} = 5.8, $J_{6a,6b}$ = 11.6), 4.31 (d, part A of AB system, H-7a, $J_{7a,7b}$ = 15.6), 4.26-4.14 (m, 4H, H-6b, H-7b, CH₂-Et, J_{5,6a} = 4.3, J_{Et} = 7.1), 3.32-3.24 (m, 2H, CH2-9), 2.27 (s, 3H, Me, Ac), 2.10 (s, 3H, Me, Ac), 1.57-1.47 (m, 2H, CH₂-10), 1.34-1.20 (m, 21 H, CH₃-Et, C₉H₁₈), 0.88 (t, 3H, CH_3 -20, J = 7.1); ¹³C NMR (100 MHz, $CDCl_3$) δ 170.9. 168.9, 168.0 (2 × CO-Ac, CO-8), 165.4 (CO), 142.1, 140.4 (C-2, C-3), 121.4 (C-4), 114.3 (C-2'), 94.1 (C-1), 67.1, 66.6 (C-5, C-7), 64.9 (C-6), 61.2 (CH₂-Et), 39.3 (C-9), 32.1, 29.8, 29.8, 29.5, 29.5, 27.1, 22.8 (C-10-C-19), 20.9, 20.9 (2 × Me, Ac), 14.3 (CH₃-Et), 14.3 (CH₃-20); HRMS: calcd for C₂₈H₄₅NO₉ [*M*+Na]⁺ 562.2992, found 562.2996.

4.1.7. General procedure for the Cul/Amberlyst A21-catalyzed cycloaddition of (*N*-propargylcarbamoyl)methyl glycosides with a terminal azide

To a solution of (*N*-propargylcarbamoyl)methyl glycoside (0.17 mmol) in dichloromethane (1.5 mL) was added azide (0.2 mmol) and Cul/Amberlyst A21 catalyst 0.8 mmol/g (17 mg, 0.08 eq.). The suspension was stirred at room. temp. overnight. After filtration of the catalyst and evaporation of the solvent, the crude was purified by column chromatography on silica gel.

4.1.7.1. {[*N***-(1-Benzyl-1***H***-1,2,3-triazol-4-yl)methyl]carbamoyl}methyl 3,4,6-tri-O-acetyl-\alpha-D-galactopyranoside (25). Cul/ Amberlyst A21-catalyzed coupling of (***N***-propargylcarbamoyl)methyl glycoside 7** (0.07 g, 0.17 mmol) with benzyl azide according to general procedure gave the triazole derivative **25** (66 mg, 71%) as a colorless oil after purification by column chromatography (from EtOAc to EtOAc/methanol, 9:1). R_f = 0.22 (EtOAc); $[\alpha]_D^{20}$ = +92 (*c* 1.3, CH₂Cl₂); H NMR (400 MHz, CDCl₃) δ 8.29 (br s, 1H, NH), 7.50 (br s, 1H, H-11), 7.38–7.31 (m, 3H, Ph), 7.26–7.21 (m, 2H, Ph), 5.44 (s, 2H, *CH*₂Ph), 5.39 (br d, 1H, H-4), 5.21 (dd, 1H, H-3, $J_{2,3}$ = 10.6, $J_{3,4}$ = 3.3), 4.92 (d, 1H, H-1, $J_{1,2}$ = 3.8), 4.55 (br d, part A of ABX system, H-9a, $J_{9a,9b}$ = 12.8), 4.32 (br d, part B of ABX system, H-9b), 4.25–4.18 (m, 2H, H-5, H-7a), 4.10–4.00 (m, 4H, H-2, H-6a, H-6b, H-7b), 2.10 (s, 3H, Me, Ac), 2.01 (s, 3H, Me, Ac), 1.97 (s, 3H, Me, Ac); 13 C NMR (100 MHz, CDCl₃) δ 170.7, 170.6, 170.2, 169.5 (3 × CO-Ac, CO-8), 134.2 (Cq, Ph), 129.3, 129.0, 128.3 (CH, Ph), 100.0 (C-1), 70.1 (C-3), 68.1, 67.6, 67.3 (C-4, C-5, C-7), 66.5 (C-2), 61.8 (C-6), 54.5 (C-1'), 34.1 (C-9), 20.9, 20.8, 20.7 (3 × Me, Ac); HRMS: calcd for C₂₄H₃₀N₄O₁₀ [*M*+Na]⁺ 557.1860, found 557.1861.

4.1.7.2. ({*N*-[1-(Hex-5-en-1-yl)-1*H*-1,2,3-triazol-4-yl]methyl}carbamoyl) methyl 3,4,6-tri-O-acetyl- α -p-galactopyranoside (26) and ({*N*-[1-(hex-5-en-1-yl)-5-iodo-1,2,3-triazol-4-yl]methyl} carbamoyl)methyl 3,4,6-tri-O-acetyl- α -p-galactopyranoside (27). Cul/ Amberlyst A21-catalyzed coupling of (*N*-propargylcarbamoyl)methyl glycoside **10** (0.099 g, 0.25 mmol) with 5-hexenyl azide according to general procedure gave the triazole **26** (96 mg, 74%) and its 5-iodo triazole derivative **27** (7 mg, 4%) as colorless oils after purification by column chromatography (from EtOAc to EtOAc/methanol, 9:1).

Data for **26**: $R_{\rm f} = 0.22$ (EtOAc); $[\alpha]_{\rm D}^{20} = +71$ (*c* 0.8, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 8.33 (t, 1H, NH), 7.55 (s, 1H, H-11), 5.78–5.66 (m, 1H, H-5'), 5.38 (br d, 1H, H-4, $J_{3,4} = 3.3$, $J_{4,5} = 1.0$), 5.21 (dd, 1H, H-3, $J_{2,3} = 10.6$), 5.05 (br d, 1H, OH), 5.01–4.92 (m, 2H, H-6'a, H-6'b), 4.91 (d, 1H, H-1, $J_{1,2} = 3.5$), 4.58 (dd, part A of ABX system, 1H, H-9a, $J_{9a,NH} = 6.3$, $J_{9a,9b} = 15.4$), 4.38–4.18 (m, 5H, H-5, H-7a, H-9b, H-1'a, H-1'b, $J_{7a,7b} = 15.6$), 4.10–4.00 (m, 4H, H-2, H-6a, H-6b, H-7b), 2.12–2.03 (m, 5H, CH₂-4', Me, Ac), 2.01 (s, 3H, Me, Ac), 1.97 (s, 3H, Me, Ac), 1.91–1.82 (m, 2H, CH₂-2'), 1.44– 1.34 (m, 2H, CH₂-3'); ¹³C NMR (100 MHz, CDCl₃) δ 170.7, 170.5, 170.2, 169.5 (3 × CO-Ac, CO-8), 144.6 (C-10), 137.7 (C-5'), 122.4 (C-11), 115.5 (C-6'), 100.0 (C-1), 70.1 (C-3), 68.1 (C-4), 67.6, (C-7), 67.2 (C-5), 66.5 (C-2), 61.8 (C-6), 50.4 (C-1'), 34.0 (C-9), 33.0 (C-4'), 29.5 (C-2'), 25.7 (C-3'), 20.9, 20.8, 20.7 (3 × Me, Ac); HRMS: calcd for C₂₃H₃₄N₄O₁₀ [*M*+Na]⁺ 549.2173, found 549.2174.

Data for **27**: $R_f = 0.48$ (EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 8.12 (t, 1H, NH), 5.91–5.66 (m, 1H, H-5'), 5.43 (dd, 1H, H-4, $J_{3,4} = 3.3$, $J_{4,5} = 1.0$), 5.26 (dd, 1H, H-3, $J_{2,3} = 10.5$), 5.09–4.96 (m, 2H, H-6'a, H-6'b), 4.93 (d, 1H, H-1, $J_{1,2} = 3.7$), 4.79 (dd, part A of ABX system, 1H, H-9a, $J_{9a,NH} = 7.6$, $J_{9a,9b} = 15.9$), 4.44 (br s, 1H, OH), 4.40–4.22 (m, 5H, H-5, H-7a, H-9b, CH_2 -1', $J_{7a,7b} = 15.6$), 4.21–4.05 (m, 4H, H-2, H-6a, H-6b, H-7b), 2.17–2.06 (m, 5H, CH_2 -4', Me, Ac), 2.05 (br s, 6H, 2 × Me, Ac), 1.97 (s, 3H, Me, Ac), 1.98–1.85 (m, 2H, CH_2 -2'), 1.52–1.38 (m, 2H, CH_2 -3'); ¹³C NMR (100 MHz, CDCl₃) δ 170.8, 170.6, 170.3, 169.3 (3 × CO-Ac, CO-8), 144.4 (C-10), 137.8 (C-5'), 115.6 (C-6'), 100.0 (C-1), 69.9 (C-3), 68.2 (C-4), 67.7, (C-7), 67.4 (C-5), 66.7 (C-2), 61.8 (C-6), 51.0 (C-1'), 34.7 (C-9), 33.1 (C-4'), 29.3 (C-2'), 25.7 (C-3'), 21.0, 20.8, 20.8 (3 × Me, Ac); HRMS: calcd for C₂₃H₃₄IN₄O₁₀ [*M*+Na]⁺ 675.1139, found 675.1140.

{[N-(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]carbam-4.1.7.3. oyl}methyl 3,4,6-tri-O-acetyl-α-D-glucopyranoside (28). CuI/ Amberlyst A21-catalyzed coupling of (N-propargylcarbamoyl)methyl glycoside 4^{18a} (0.115 g, 0.29 mmol) with benzyl azide according to general procedure gave the triazole derivative 28 (92 mg, 60%) as a colorless oil after purification by column chromatography (from EtOAc to EtOAc/methanol, 9:1); *R*_f = 0.21 (EtOAc); $[\alpha]_{D}^{20}$ = +64 (c 0.9, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 8.24 (br t, 1H, NH), 7.45 (br s, 1H, H-11), 7.40-7.33 (m, 3H, Ph), 7.29-7.23 (m, 2H, Ph), 5.47 (s, 2H, CH₂Ph), 5.34 (br d, 1H, H-3, $J_{2,3} = J_{3,4} = 10.0$), 5.02 (t, 1H, H-4, $J_{3,4} = J_{4,5}$), 4.86 (d, 1H, H-1, $J_{1,2}$ = 3.8), 4.69 (dd, 1H, H-9a, $J_{9a,NH}$ = 7.3, $J_{9a,9b}$ = 15.5), 4.35–4.19 (m, 3H, H-6a, H-7a, H-9b), 4.09-4.00 (m, 3H, H-5, H-6b, H-7b), 3.84 (dd, 1H, H-2), 2.08 (s, 3H, Me, Ac), 2.06 (s, 3H, Me, Ac), 2.02 (s, 3H, Me, Ac); ¹³C NMR (100 MHz, CDCl₃) δ 171.2, 170.8, 169.8, 169.5 (3 × CO-Ac, CO-8), 145.0 (C-10), 134.2 (Cq, Ph), 129.3, 129.1, 128.3 (CH, Ph), 122.1 (C-11), 99.5 (C-1), 72.8 (C-3), 70.3 (C-2), 68.3, 68.2, 67.7 (C-4, C-5, C-7), 62.0 (C-6), 54.5 (C-1'), 34.0 (C-9), 21.0 20.9, 20.8 (3 × Me, Ac); HRMS: calcd for $C_{24}H_{30}N_4O_{10}$ [*M*+Na]⁺ 557.1860, found 557.1859.

4.1.7.4. {[*N*-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl]carbamoyl}methyl 3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (29) and {[*N*-(1-benzyl-5-iodo-1,2,3-triazol-4-yl)methyl]carbamoyl}methyl 3,4,6tri-*O*-acetyl-β-D-glucopyranoside (30). Cul/Amberlyst A21catalyzed coupling of (*N*-propargylcarbamoyl)methyl glycoside 7^{18b} (51 mg, 0.13 mmol) with benzyl azide according to general procedure gave the triazole 29 (52 mg, 77%) as a white solid and its 5-iodo triazole derivative 30 (3 mg, 4%) a colorless oil after purification by column chromatography (from EtOAc to EtOAc/MeOH, 9:1).

Data for **29**: $R_f = 0.24$ (EtOAc); mp 147–149 °C; $[\alpha]_D^{20} = +2$ (*c* 0.5, CH_2Cl_2); ¹H NMR (400 MHz, CDCl₃) δ 8.01 (t, 1H, NH, J = 5.8), 7.47 (br s, 1H, H-11), 7.37-7.32 (m, 3H, Ph), 7.26-7.22 (m, 2H, Ph), 5.48 (d, 1H, OH, J = 5.8), 5.45 (br s, 2H, CH₂Ph), 5.14 (d, 1H, H-3, $J_{2,3} = J_{3,4} = 9.6$), 4.99 (t, 1H, H-4, $J_{3,4} = J_{4,5}$), 4.51–4.37 (m, 3H, H-1, CH_2 -9, $J_{1,2}$ = 7.8, $J_{9a,9b}$ = 15.4), 4.34 (d, part A of AB system, 1H, H-7a, $J_{7a,7b}$ = 15.6), 4.25 (dd, part A of ABX system, 1H, H-6a, J_{5,6a} = 4.8, J_{6a,6b} = 12.3), 4.12 (d, part B of AB system, 1H, H-7b), 4.07 (dd, part B of ABX system, 1H, H-6b, J_{5.6b} = 2.0), 3.69 (ddd, 1H, H-5), 3.61 (ddd, 1H, 2H), 2.05 (s, 3H, Me, Ac), 2.01 (s, 3H, Me, Ac), 2.00 (s, 3H, Me, Ac); 13 C NMR (100 MHz, CDCl₃) δ 170.9, 170.8, 169.7, 169.6 (3 × CO-Ac, CO-8), 145.5 (C-10), 134.2 (Cq, Ph), 129.3, 129.0, 128.3 (CH, Ph), 122.6 (C-11), 103.1 (C-1), 74.9 (C-3), 72.1 (C-5), 71.8 (C-2), 68.8 (C-7), 68.3 (C-4), 62.0 (C-6), 54.4 (C-1'), 34.1 (C-9), 20.9, 20.8, 20.7 (3 × Me, Ac); HRMS: calcd for C₂₄H₃₀N₄O₁₀ [*M*+Na]⁺ 557.1860, found 557.1861.

Data for **30**: $R_f = 0.41$ (EtOAc); ¹H NMR (400 MHz, CDCl₃) δ 7.81 (br t, 1H, NH), 7.40–7.32 (m, 3H, Ph), 7.30–7.23 (m, Ph), 5.58 (br s, 2H, CH₂Ph), 5.12 (d, 1H, H-3, $J_{2,3} = J_{3,4} = 9.6$), 5.03 (t, 1H, H-4, $J_{3,4} = J_{4,5}$), 4.61 (dd, part A of ABX system, 1H, H-9a, $J_{9a,NH} = 5.5$, $J_{9a,9b} = 15.4$), 4.49–4.35 (m, 3H, H-1, H-9b, H-7a $J_{1,2} = 8.1$, $J_{7a,7b} = 16.1$, $J_{9a,9b} = 15.4$), 4.29–4.18 (m, 1H, H-6a, H-7b, $J_{5,6a} = 4.8$, $J_{6a,6b} = 12.3$), 4.09 (dd, part B of ABX system, 1H, H-6b, $J_{5,6b} = 2.0$), 3.68 (ddd, 1H, H-5), 3.62 (dd, 1H, 2H), 2.09 (s, 3H, Me, Ac), 2.07 (s, 3H, Me, Ac), 2.03 (s, 3H, Me, Ac); ¹³C NMR (100 MHz, CDCl₃) δ 171.1, 170.8, 169.7, 169.4 (3 × CO-Ac, CO-8), 133.9 (Cq, Ph), 129.1, 128.8, 128.1 (CH, Ph), 103.1 (C-1), 75.3 (C-3), 72.3 (C-5), 72.1 (C-2), 68.7 (C-7), 68.1 (C-4), 62.0 (C-6), 54.6 (C-1'), 34.9 (C-9), 21.0, 20.9, 20.8 (3 × Me, Ac); HRMS: calcd for C₂₄H₂₉IN₄O₁₀ [*M*+Na]⁺ 683.0827, found 683.0827.

4.1.7.5. ({N-Allyl, N-[1-(hex-5-en-1-yl)-1H-1,2,3-triazol-4yl]methyl}carbamoyl)methyl 2,3,4,6-tetra-O-allyl-α-p-galactopyranoside (31) and ({N-Allyl, N-[1-(hex-5-en-1-yl)-1H-1,2,3triazol-4-yl]methyl}carbamoyl)methyl 2,4,6-tri-O-allyl-α-Dgalactopyranoside (32). To a solution of compound 26 (44 mg, 0.08 mmol) in dry DMF (1.2 mL) was added NaH (0.42 mmol, 60%, 17 mg). After a few minutes, allyl bromide (0.42 mmol, 36 µL) was added and the mixture was stirred at 50 °C for 1 h. Water was added (8 mL) and the aqueous phase was extracted with EtOAc (3 \times 3 mL). The combined organic layers were washed with water and dried with Na₂SO₄. After filtration and evaporation of the solvent, the residue was purified by column chromatography (from EtOAc/pentane, 7:3 to EtOAc) to afford 31 (16 mg, 32%) and **32** (9 mg, 20%) as colorless oils.

Data for **31**: $R_f = 0.56$ (EtOAc/pentane, 7:3); $[\alpha]_D^{20} = +19$ (c 1.2, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.58 (s, 1H, H-11), 6.02– 5.67 (m, 6H, 5 × CH allylic, H-5'), 5.37–5.08 (m, 11H, H-1, 5 × =CH₂ allylic), 5.06–4.93 (m, 2H, =CH₂-6'), 4.60 (d, part A of AB system, H-9a, $J_{9a,9b} = 14.9$), 4.53 (d, part A of AB system, H-9b), 4.44–3.83 (m, 16H, 5 × CH₂ allylic, H-2, H-5, CH₂-7, CH₂-1', $J_{1,2} = 3.7$), 3.81 (br d, 1H, H-4, $J_{3,4} = 3.1$), 3.75 (dd, 1H, H-3, $J_{3,4} = 2.9$, $J_{2,3} = 10.1$), 3.62–3.45 (m, 2H, CH₂-6, $J_{5,6a} = 6.4$, $J_{5,6b} = 6.4$, $J_{6a,6b} = 9.3$), 2.14–2.04 (m, 2H, CH₂-4'), 1.96–1.82 (m, 2H, CH₂-2'), 1.48–1.35 (m, 2H, CH₂-3'); ¹³C NMR (100 MHz, CDCl₃) δ 168.8 (CO-8), 144.2 (C-10), 137.9, 135.5 135.3, 135.3, 134.6, 132.6 (C-5', 5 × CH allylic), 123.4 (C-11), 117.5, 117.3, 117.2, 117.0, 116.3 (5 × =CH₂ allylic) 115.5 (C-6'), 97.2 (C-1), 78.0 (C-3), 76.0 (C-2), 75.1 (C-4), 74.2 (CH₂ allylic-4), 72.5 (CH₂ allylic-6), 72.1, 72.1 (CH₂ allylic-2, CH₂ allylic-3), 70.0 (C-5), 69.0 (C-6), 64.5 (C-7), 50.3 (C-1'), 49.5 (N-CH₂ allylic), 41.0 (C-9), 33.1 (C-4'), 29.7 (C-2'), 25.8 (C-3'); HRMS: calcd for C₃₂H₄₈N₄O₇ [*M*+Na]⁺ 623.3421, found 623.3423.

Data for **32**: $R_{\rm f} = 0.23$ (EtOAc/pentane, 7:3); $[\alpha]_{\rm D}^{20} = +32$ (c 0.7, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.58 (s, 1H, H-11), 6.04-5.58 (m, 5H, $4 \times$ CH allylic, H-5'), 5.35–5.11 (m, 9H, H-1, $4 \times =$ CH₂ allylic), 5.07-4.94 (m, 2H, =CH₂-6'), 4.62 (d, part A of AB system, H-9a, *J*_{9a,9b} = 15.1), 4.53 (d, part A of AB system, H-9b), 4.40–3.83 (m, 14H, $4 \times CH_2$ allylic, H-3, H-5, CH_2 -7, CH_2 -1'), 3.82 (br d, 1H, H-4, $J_{3,4}$ = 3.1), 3.73 (dd, 1H, H-2, $J_{1,2}$ = 3.7, $J_{2,3}$ = 10.3), 3.62–3.50 (m, 2H, CH_2 -6, $J_{5,6a}$ = 6.5, $J_{5,6b}$ = 6.5, $J_{6a,6b}$ = 9.3), 2.17–2.02 (m, 2H, CH2-4'), 1.99-1.82 (m, 2H, CH2-2'), 1.52-1.34 (m, 2H, CH2-3'); ¹³C NMR (100 MHz, CDCl₃) δ 168.8 (CO-8), 144.1 (C-10), 137.9, 135.1, 134.8, 134.5, 132.4 (C-5', 4 × CH allylic), 123.4 (C-11), 117.9, 117.6, 117.4, 117.3 ($4 \times = CH_2$ allylic) 115.5 (C-6'), 96.1 (C-1), 76.5 (C-4, C-2), 74.5 (CH₂ allylic-4), 72.5 (CH₂ allylic-6), 71.3 (CH₂ allylic-2), 69.9, 69.9 (C-3, C-5), 69.0 (C-6), 64.1 (C-7), 50.4 (C-1'), 49.5 (N-CH₂ allylic), 41.0 (C-9), 33.1 (C-4'), 29.7 (C-2'), 25.8 (C-3'); HRMS: calcd for C₂₉H₄₄N₄O₇ [M+Na]⁺ 583.3108, found 583.3105.

4.1.7.6. {[N-(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]carbamoyl} methyl 3,6-di-O-acetyl-4-deoxy-α-D-glycero-hex-3-enopyranosid-2-ulose (33). DMSO/Ac₂O oxidation of compound 28 (88 mg, 0.17 mmol) according to general procedure gave the corresponding 3-enopyranosid-2-ulose 33 (47 mg, 60%) as a colorless oil after purification by column chromatography (from EtOAc/pentane, 4:1 to EtOAc). $R_{\rm f} = 0.40$ (EtOAc); $[\alpha]_{\rm D}^{20} = +6$ (c 0.4, CH₂Cl₂); ¹H NMR (300 MHz, CDCl₃) δ 7.47 (br s, 1H, H-11), 7.40–7.33 (m, 3H, Ph), 7.29-7.23 (m, 2H, Ph), 7.04 (br t, 1H, NH), 6.64 (d, 1H, H-4, $J_{4.5}$ = 1.7), 5.49 (br s, 2H, CH₂Ph), 5.02 (s, 1H, H-1), 4.95 (td, 1H, H-5), 4.57–4.51 (m, 2H, CH₂-9) 4.41 (dd, part A of ABX system, 1H, H-6a, *J*_{5.6a} = 5.3, *J*_{6a.6b} = 11.7), 4.31 (d, part A of AB system, 1H, H-7a, *J*_{7a,7b} = 15.1), 4.25–4.14 (m, 2H, H-6b, H-7b, *J*_{5,6b} = 4.5), 2.25 (s, 3H, Me, Ac), 2.10 (s, 3H, Me, Ac); 13 C NMR (100 MHz, CDCl₃) δ 181.6 (CO), 170.7, 168.1, 167.9 (2 × CO-Ac, CO-8), 141.8 (C-4), 134.6 (Cq, Ph), 133.2 (C-3), 129.2, 128.9, 128.2 (CH, Ph), 98.1 (C-1), 68.4, 68.3 (C-5, C-7), 64.3 (C-6), 54.3 (C-1'), 34.7 (C-9), 20.8, 20.4 (2 × Me, Ac); HRMS: calcd for $C_{22}H_{24}N_4O_8$ [*M*+Na]⁺ 495.1492, found 495.1493.

4.1.7.7. {[N-(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]carbamoyl} methyl 3,6-di-O-acetyl-2,4-dideoxy-2-C-[(E)-(ethoxycarbonyl) methylene]-α-*p-glycero*-hex-3-enopyranoside (34). CuI/ Amberlyst A21-catalyzed coupling of (N-propargylcarbamoyl)methyl glycoside 22 (20 mg, 0.05 mmol) with benzyl azide according to general procedure gave the triazole derivative 34 (23 mg, 87%) as a white solid after purification by column chromatography (from EtOAc/pentane, 7:3 to EtOAc). $R_f = 0.43$ (EtOAc); mp 172–174 °C; $[\alpha]_D^{20}$ = +10 (*c* 0.2, CH₂Cl₂); ¹H NMR (400 MHz, CDCl₃) δ 7.57 (br t, 1H, NH), 7.48 (s, 1H, H-11), 7.39–7.32 (m, 3H, Ph), 7.29-7.23 (m, 2H, Ph), 6.36 (s, 1H, H-1), 5.93 (dd, 1H, H-4, J_{2',4} = 0.8, J_{4.5} = 2.0), 5.89 (br d, 1H, H-2'), 5.48 (s, 2H, CH₂Ph), 4.69 (ddd, 1H, H-5), 4.60 (dd, part A of ABX system, 1H, H-9a, $J_{9a,NH} = 6.3$, $J_{9a,9b} = 15.4$), 4.50 (dd, part B of ABX system, 1H, H-9b, $J_{9a,NH} = 6.0$), 4.37–4.27 (m, 2H, H-6a, H-7a, $J_{5,6a} = 5.8$, J_{6a,6b} = 11.6, J_{7a,7b} = 15.6), 4.24 (d, part B of AB system, 1H, H-7b), 4.18-4.09 (m, 3H, H-6b, CH₂-Et, J_{5,6b} = 4.0, J_{Et} = 7.1), 2.26 (s, 3H, Me, Ac-3), 2.09 (s, 3H, Me, Ac-6), 1.25 (t, 1H, CH₃-Et); ¹³C NMR (100 MHz, CDCl₃) δ 170.9, 169.2, 168.0 (2 × CO-Ac, CO-8), 165.4

(CO), 145.4 (C-10), 142.0, 140.1 (C-2, C-3), 134.7 (Cq, Ph), 129.3, 128.9, 128.2 (CH, Ph), 122.5 (C-11), 121.3 (C-4), 114.4 (C-2'), 94.3 (C-1), 67.1, 66.8 (C-5, C-7), 68.7 (C-7), 64.9 (C-6), 61.3 (CH₂-Et), 54.3 (C-1'), 34.7 (C-9), 21.0, 20.9 ($2 \times$ Me, Ac), 14.3 (CH₃-Et); HRMS: calcd for C₂₆H₃₀N₄O₉ [*M*+Na]⁺ 565.1910, found 565.1914.

4.1.8. General procedure for deacetylation of compounds 25, 26, 28, and 29

A solution of 2,3,4,6-tetra-O-acetylated glycoside (0.03 mmol) in $CH_3OH/H_2O/NEt_3$ (8:1:1, 2 mL) was stirred overnight at 40 °C. After evaporation of the solvents under vacuum, the residue was purified by column chromatography.

4.1.8.1. {[N-(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]carbamoyl} methyl- α -p-galactopyranoside (35). Deacetvlation (CH₃OH/ H₂O/NEt₃) of glycoside **25** (19 mg, 0.036 mmol), according to general procedure afforded compound **35** (12 mg, 82%) as a colorless oil after purification by column chromatography (EtOAc/MeOH, 4:1). $R_{\rm f} = 0.23$ (EtOAc/methanol, 4:1); $[\alpha]_{\rm D}^{20} = +61$ (c 1, MeOH/ CH₂Cl₂, 1:1); ¹H NMR (400 MHz, MeOD) δ 7.87 (br s, 1H, H-11), 7.42-7.28 (m, 3H, Ph), 5.57 (s, 2H, CH₂Ph), 4.85 (d, 1H, H-1, $J_{1,2}$ = 3.5), 4.51 (s, 2H, CH₂-9), 4.21 (d, part A of AB system, H-7a, $I_{7a,7b}$ = 15.9), 4.03 (d, part B of AB system, H-7b), 3.88 (br d, 1H, H-4, $J_{3,4}$ = 3.3), 3.84–3.63 (m, 5H, H-2, H-3, H-5, H-6a, H-6b, $J_{2,3}$ = 10.3, $J_{5,6b}$ = 5.0, $J_{6a,6b}$ = 11.3); ¹³C NMR (100 MHz, MeOD) δ 172.3 (CO-8), 136.7 (Cq, Ph), 130.0, 129.6, 129.2 (CH, Ph), 124.3 (C-11), 101.2 (C-1), 73.1 (C-3), 71.3 (C-5), 71.0 (C-4), 70.0 (C-2), 67.9 (C-7), 62.8 (C-6), 55.0 (C-1'), 35.2 (C-9); HRMS: calcd for C₁₈H₂₄N₄O₇ [*M*+Na]⁺ 431.1543, found 431.1544.

4.1.8.2. ({*N*-[1-(Hex-5-en-1-yl)-1*H*-1,2,3-triazol-4-yl]methyl} carbamoyl)methyl- α -D-galactopyranoside (36). Deacetvlation (CH₃OH/H₂O/NEt₃) of glycoside 26 (17 mg, 0.032 mmol), according to general procedure afforded compound 36 (12 mg, 95%) as a colorless oil after purification by column chromatography (EtOAc/MeOH, 4:1). $[\alpha]_D^{20} = +33$ (*c* 0.6, MeOH/CH₂Cl₂, 1:1); *R*_f = 0.32 (EtOAc/methanol, 5:1); ¹H NMR (400 MHz, MeOD) δ 7.88 (br s, 1H, H-11), 5.88-5.71 (m, 1H, H-5'), 5.07-4.91 (m, 2H, H-6'a, H-6'b), 4.86 (d, 1H, H-1), 4.52 (s, 2H, CH₂-9), 4.39 (t, 2H, CH₂-1'), 4.24 (d, part A of AB system, H-7a, $J_{7a,7b}$ = 15.5), 4.05 (d, part B of AB system, H-7b), 3.89 (br d, 1H, H-4, J_{3,4} = 3.1, J_{4,5} = 1.0), 3.85–3.64 (m, 5H, H-2, H-3, H-5, H-6a, H-6b, $J_{1,2} = 3.1$, $J_{2,3} = 10.1$, $J_{5,6b} = 5.3$, $I_{6a.6b} = 11.1$), 2.14–2.06 (m, 5H, CH₂-4'), 1.98–1.84 (m, 2H, CH₂-2'), 1.46–1.35 (m, 2H, CH₂-3'); ¹³C NMR (100 MHz, MeOD) δ 172.3 (CO-8), 146.0 (C-10), 139.2 (C-5'), 124.2 (C-11), 115.5 (C-6'), 101.3 (C-1), 73.1 (C-3), 71.3 (C-5), 71.0 (C-4), 70.0 (C-2), 68.0 (C-7), 62.8 (C-6), 51.2 (C-1'), 35.2 (C-9), 34.1 (C-4'), 30.7 (C-2'), 26.8 (C-3'); HRMS: calcd for C₁₇H₂₈N₄O₇ [*M*+Na]⁺ 423.1856, found 423.1858.

4.1.8.3. {[*N*-(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methyl]carbamoyl} methyl- α -D-glucopyranoside (37). Deacetylation (CH₃OH/ H₂O/NEt₃) of glycoside 28 (17 mg, 0.032 mmol), according to general procedure afforded compound 37 (12 mg, 92%) as a colorless oil after purification by column chromatography (EtOAc/MeOH, 4:1). $R_{\rm f} = 0.24$ (EtOAc/methanol, 4:1); $[\alpha]_{\rm D}^{20} = +57$ (c 1.1, MeOH/ CH₂Cl₂, 1:1); ¹H NMR (400 MHz, MeOD) δ 7.88 (br s, 1H, H-11), 7.41-7.29 (m, 3H, Ph), 5.57 (s, 2H, CH₂Ph), 4.81 (d, 1H, H-1, $J_{1,2}$ = 3.8), 4.51 (s, 2H, CH₂-9), 4.22 (d, part A of AB system, H-7a, J_{7a.7b} = 15.6), 4.03 (d, part B of AB system, H-7b), 3.80 (dd, part A of ABX system, H-6a, J_{5,6a} = 2.2, J_{6a,6b} = 11.8), 3.68–3.61 (m, 2H, H-3, H-6b), 3.54 (ddd, 1H, H-5, *J*_{4,5} = 9.8, *J*_{5,6b} = 5.8), 3.43 (dd, 1H, H-2, $J_{2,3}$ = 9.6), 3.33 (dd, 1H, H-4, $J_{3,4}$ = 9.1); ¹³C NMR (100 MHz, MeOD) & 172.2 (CO-8), 146.3 (C-10), 136.7 (Cq, Ph), 130.0, 129.6, 129.2 (CH, Ph), 124.3 (C-11), 101.0 (C-1), 74.9 (C-3), 74.4 (C-5), 73.3 (C-2), 71.6 (C-4), 67.8 (C-7), 62.5 (C-6), 55.0 (C-1'), 35.2 (C-

9); HRMS: calcd for $C_{18}H_{24}N_4O_7$ [*M*+Na]⁺ 431.1543, found 431.1541.

4.1.8.4. {[N-(1-Benzyl-1H-1,2,3-triazol-4-yl)methyl]carbamoyl}methyl-β-D-glucopyranoside (38). Deacetylation (CH₃OH/H₂O/NEt₃) of glycoside **29** (16 mg, 0.03 mmol), according to general procedure afforded compound 38 (11 mg, 90%) as a white solid after purification by column chromatography (EtOAc/ MeOH, 4:1). *R*_f = 0.24 (EtOAc/methanol, 4:1); mp 154–155 °C; $[\alpha]_{D}^{20} = -11 (c \, 0.8, \text{MeOH/CH}_2\text{Cl}_2, 1:1); ^{1}\text{H NMR} (400 \text{ MHz}, \text{MeOD}) \delta$ 7.87 (br s, 1H, H-11), 7.41-7.29 (m, 5H, Ph), 5.57 (s, 2H, CH₂Ph), 4.53 (d, part A of AB system, H-9a, $J_{7a,7b}$ = 15.4), 4.48 (d, part B of AB system, H-9b), 4.35-4.28 (m, 2H, H-1, H-7a, $J_{1,2} = 7.8$, J_{7a,7b} = 15.9), 4.17 (d, part B of AB system, H-7b), 3.83 (dd, part A of ABX system, H-6a, $J_{5,6a}$ = 1.8, $J_{6a,6b}$ = 12.1), 3.65 (dd, part A of ABX system, H-6b, $J_{5,6b}$ = 5.3) 3.89–3.21 (m, 4H, H-2, H-3, H-4, H-5); ¹³C NMR (100 MHz, MeOD) δ 172.4 (CO-8), 136.7 (Cq, Ph), 130.0, 129.6, 129.2 (CH, Ph), 124.3 (C-11), 104.7 (C-1), 78.2 (C-5), 77.8 (C-3), 74.9 (C-2), 71.4 (C-4), 69.5 (C-7), 62.5 (C-6), 55.0 (C-1'), 35.2 (C-9); HRMS: calcd for C₁₈H₂₄N₄O₇ [*M*+Na]⁺ 431.1543, found 431.1543.

4.2. Biological assays

4.2.1. Antimicrobial activity

The antibacterial and antifungal activity of compounds 13-15, 18, 21-24 and 25-26, 28-29, and 31-38 was evaluated using the paper disk diffusion method according to the standard procedure CLSI (Clinical Laboratory Standards Institute/National Committee for Clinical Laboratory Standards).³⁶ The following bacteria and fungi were used in the tests: E. faecalis (ATCC 7080), E. coli (ATCC 25922), L. monocytogenes (ATCC19115), S. enteritidis (ATCC 13076), S. aureus (ATCC 6538), B. cereus (ATCC 11778), B. subtilis (ATCC 6633), A. niger (ATCC 16404) B. cinerea (ESAS), C. albicans (ATCC 10231), P. aurantiogriseum (ATCC 16025). The overnight cultures of the microorganisms were spread over the appropriate media, nutrient agar for all bacteria except *Listeria*, where tryptone sova agar was used. Potato dextrose agar was used for fungi. Paper disks of 6.4 mm were placed on the agar and a solution of each substance (300 µg) in DMSO (15 µL) was applied on each disk. Chloramphenicol (for B. cereus, B. subtillis, E. faecalis, E. coli, L. monocytogenes, S. enteritidis, and S. aureus) and actidione and amphotericine B (for A. niger, B. cinerea, C. albicans, and P. aurantiogriseum) were used as positive controls and DMSO was used as negative control. Bacteria were incubated at 37 °C for 24 h and fungi at 25 °C for 24-48 h. After incubation, the plates presented a biomass lawn and, when applicable, the nearest diameter of the inhibition zones formed was measured. Results were the average of two replicates.

The antimicrobial activity was classified according to the diameter of inhibition zones (\emptyset), as follows: very strong activity, $\emptyset \ge 26 \text{ mm}$, +++++; strong activity, $22 \text{ mm} \le \emptyset < 26 \text{ mm}$, ++++; good activity, $18 \text{ mm} \le \emptyset < 22 \text{ mm}$, +++; moderate activity, $14 \text{ mm} \le \emptyset < 18 \text{ mm}$, ++; weak activity, $12 \text{ mm} \le \emptyset < 14 \text{ mm}$, +; no activity, $\emptyset < 12 \text{ mm}$, –.

4.2.2. Acute toxicity

Acute cytotoxicity measurements were performed by the MTT method.³⁸ The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to quantify metabolically viable cells in all samples. Adherent cells (mouse HII4E hepatoma cells) were seeded onto 96-well plates, allowed to attach for 24 h and exposed to the test compound for the following 24 h. Positive control (hydrogen peroxide) and negative control (DMSO) were also included. At 48 h of culture MTT was added to the cells at a final concentration of 0.5 mg/mL, followed by an incubation period of 3 h to allow the formazan crystals to form. After incubation, medium was removed, cells were washed twice to remove traces of medium and un-metabolized MTT, and DMSO (100 μ L) was added to each well. Solubilization of formazan crystals was performed by agitation in a 96-well plate shaker for 20 min at room temperature. Absorbance of each well was quantified at 550 nm using 620 nm as reference wavelength on a scanning multiwall spectrophotometer (automated plate reader).

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