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Synthesis of Unprecedented Sulfonylated Phosphono-*exo*-Glycals Designed as Inhibitors of the Three Mycobacterial Galactofuranose Processing Enzymes

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Abstract: This study reports a new methodology to synthesize *exo*-glycals bearing both a sulfone and a phosphonate. This synthetic strategy provides a way to generate *exo*-glycals displaying two electron-withdrawing groups and was applied to eight different carbohydrates from the furanose and pyranose series. The *Z*/*E* configurations of these tetrasubstituted enol ethers could be ascertained using NMR spectroscopic techniques. Deprotection of an *exo*-glycal followed by an UMP (uridine monophosphate) coupling generated two new UDP (uridine diphosphate)-galactofuranose analogues. These two *Z*/*E* isomers were evaluated as inhibitors of UGM, Glf*T*1, and Glf*T*2, the three mycobacterial galactofuranose processing enzymes. Molecule **46-(*E*)** is the first characterized inhibitor of Glf*T*1 reported to date and was also found to efficiently inhibit UGM in a reversible manner. Interestingly, Glf*T*2 showed a better affinity for the (*Z*) isomer. The three enzymes studied in the present work are not only interesting because, mechanistically, they are still the topic of intense investigations, but also because they constitute very important targets for the development of novel antimycobacterial agents.

Introduction

Despite the availability of potent drugs and vaccines, tuberculosis (TB) remains a major threat for public health worldwide, mainly because of the emergence of multi-drug resistant strains.[1] In this context, the inhibition of the mycobacterial cell wall biosynthesis of *Mycobacterium tuberculosis*, the causative agent of tuberculosis, has received significant attention.[2] The main mycobacterial cell wall component is the mycolyl–arabinogalactan–peptidoglycan (mAPG) complex, which is composed of an arabinogalactan (AG) polysaccharide linked to the peptidoglycan by a disaccharide linker. About two thirds of the arabinan chains are covalently linked to mycolic acids that enhance the cellular resistance against antibiotics. As a consequence, this mAPG constitutes a true Achilles heel of the bacterium as inhibition of its biosynthesis prevents *M. tuberculosis* survival in the infected host. Therefore, the AG is still a major target for the development of antitubercular drugs because of its essential role for the viability of mycobacteria.[3] The best-characterized enzymes involved in the AG biosynthesis are UDP (uridine diphosphate)-galactopyranose mutase (UGM), two galactofuranosyltransferases (Glf*T*s), and several arabinosyltransferases (AraTs).[2a,4] UGM is a flavoenzyme with a flavine adenine dinucleotide (FAD) cofactor that plays an unprecedented catalytic role as a nucleophile in the isomerization of UDP-galactopyranose (**1**, UDP-Gal*p*) into its furanose isomer UDP-galactofuranose (**2**, UDP-Gal*f*, Figure 1).[5] The polymeric galactan is constructed by two Glf*T*s that catalyze the glycosylation reactions.[6] The first transferase (Glf*T*1)[7] adds the two initial galactofuranose (Gal*f*) units to the disaccharide linker, while the second transferase (Glf*T*2)[3a,8] catalyzes a polymerization[9] that yields a final galactan composed of 30–35 galactofuranose moieties (Figure 1 A).[3d,10] The inhibition of UGM and the Glf*T*s would prevent the biosynthesis of AG and, thus, weaken the *M. tuberculosis* cell wall. Several attempts have been made to inhibit UGM,[2a,11] but only very few inhibition studies of Glf*T*1 and Glf*T*2 have been described so far.[2a,12]

In previous studies, we discovered that *exo*-glycals such as **3** and **4-(*Z*,*E*)** could inhibit UGM and/or Glf*T*2 (Figure 1 B).[13]

The principle used in the design of these compounds is that the sp^2 hybridization of the anomeric carbon of the phosphonylated *exo*-glycals **3**, **4**, or **6** may lock the molecule in a confor-

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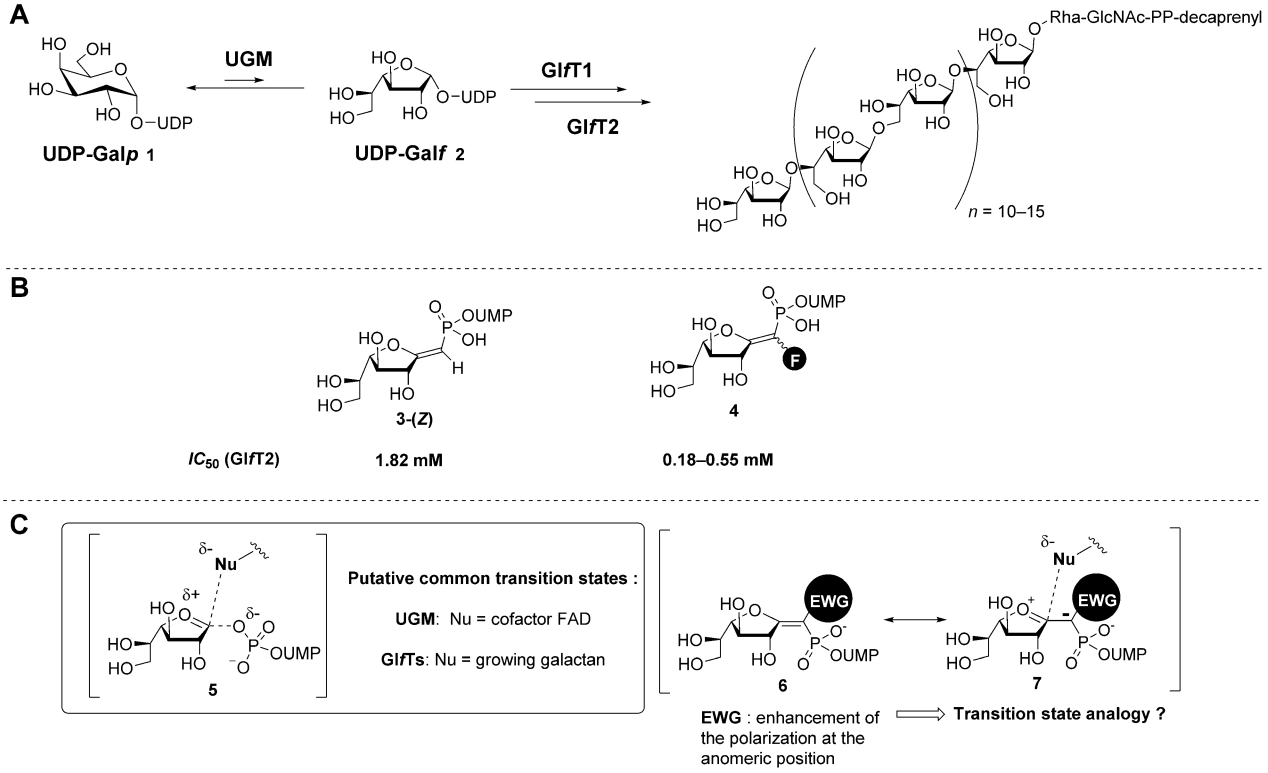


Figure 1. A) The mycobacterial galactan biosynthesis. B) Structures of previous GlfT2 (GlfT = galactofuranosyltransferase) inhibitors **3** and **4**. C) Design principle of the present work.

mation mimicking the putative oxacarbenium-ion-like transition state **5** (Figure 1 C).

It is generally believed that the transition states of glycosyltransferase-catalyzed reactions have a substantial cationic character at the anomeric carbon.[14] We, thus, consider that the putative structure **5** may mimic the transition states of both GlfT1 and GlfT2. Moreover, several mechanistic studies have suggested that the transition state of the UGM-catalysed isomerization also exhibits a partial positive charge at the anomeric position.[5c] Therefore, these three Gal*f*-processing enzymes could share some common conformational and electronic features in their respective transition states. Based on this analysis, we became interested in *exo*-glycals of the general structure **6** (Figure 1 C); the presence of an electron-withdrawing group (EWG) would likely enhance the polarization of the conjugated exocyclic enol ether, which might improve the interactions between the substrate analogues and the enzyme and/or the incoming nucleophile (the cofactor for UGM or the growing galactan for the two Glf*T*s). Our preliminary results with fluorinated *exo*-glycals **4** support this hypothesis.[13b]

To explore this concept, we describe the synthesis of sulfonylated *exo*-glycals and their inhibition properties not only against UGM and Glf*T*2, but also against the poorly studied Glf*T*1 enzyme. The sulfonyl group was selected because of its remarkable electron-withdrawing properties, as evidenced by the unique Michael acceptor ability of vinyl sulfones.[15]

This study will begin with a description of a new general methodology to generate sulfonylated tetrasubstituted *exo*-glycals. The scope of this transformation with regards to substrate and protecting groups will be discussed. Then, the detailed synthesis of the UDP-Gal*f* analogues will be shown, followed by their biochemical characterization using inhibition assays.

Results and Discussion

The main methods to synthesize *exo*-glycals are Wittig-like olefinations,[16] the Ramberg–Bäcklund rearrangement,[17] the modified Julia olefination,[18] and the stepwise addition–elimination of sugar lactones.[19]

Recently, different functionalizations,[20] such as the Sonogashira,[21] Suzuki,[22] and Stille cross-coupling reactions,[23] have allowed the preparation of tetrasubtituted *exo*-glycals. However, to our knowledge, there is no general method to synthesize tetrasubstituted *exo*-glycals bearing two electron-withdrawing groups. In fact, the methodology developed by Lin,[19] which consists of a nucleophilic addition of a lithiated methyl-phosphonate to a sugar lactone, does not yield the desired addition product when a lithiated phosphonate bearing an electron-withdrawing group is used (Figure 2, Path A). This lack of reactivity is likely due to the poor nucleophilicity of the phosphonate.

Given the problems encountered in the direct addition of lithiated phosphonates bearing an electron-withdrawing group, we explored another strategy (Figure 2, Path B) that consists of an addition of a lithiated phosphonate bearing an

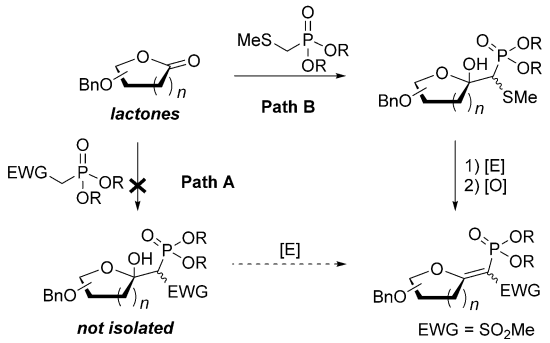
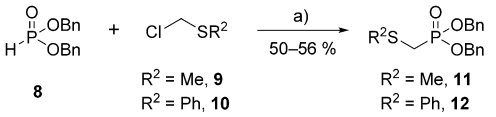


Figure 2. Addition of a lithiated phosphonate bearing an electron-withdrawing group. [E] = elimination, [O] = oxidation.

electron-donating group SR followed by an oxidation to ultimately give the desired sulfonyl moiety. To develop this new approach, we selected the commercially available tribenzylated arabinolactone **14** as a model substrate (Table 1).

The phosphonylated thioethers **11** and **12** required for this study were obtained through a classical Michaelis–Becker reaction as depicted in Scheme 1, while the diethyl (thiomethyl)-methyl phosphonate **13** is commercially available.

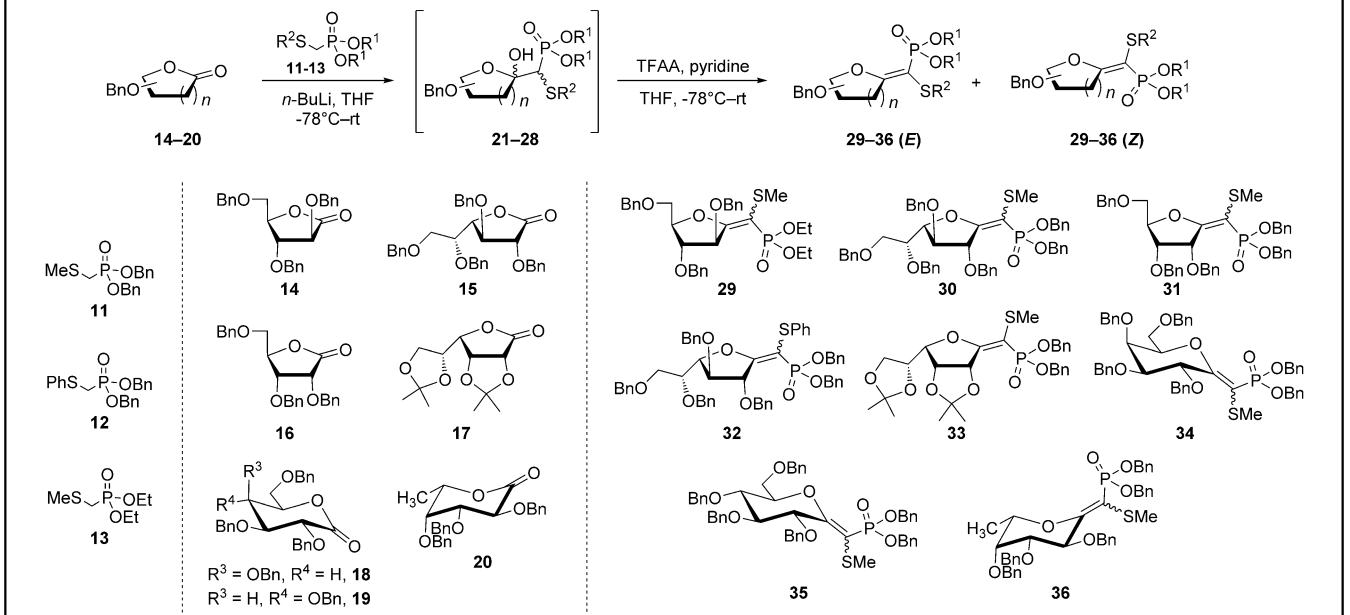


Scheme 1. Reagents and conditions: a) NaH, THF, 40 °C, 16–24 h.

As an initial experiment, the phosphonate **13** was lithtiated and added to the perbenzylated lactone **14** to afford the lactol **21** (see scheme in Table 1). The following elimination was achieved by treating **21** with pyridine and trifluoroacetic anhydride (TFAA) to give the *exo*-glycal **29-(*Z*,*E*)** in 80% yield over two steps in a 70:30 *Z*/*E* ratio (Table 1, Entry 1).

Having established the optimal conditions for this stepwise addition–elimination sequence, the scope of this transformation was examined with various lactones. All starting lactones **15**–**20** were prepared following procedures described in the literature.[24] The results gathered in Table 1 show that, in all cases, satisfactory yields were obtained. Moreover, we noticed that the reaction times of the addition and elimination steps depend on the nature of the lactone as well as the phosphonate reagents used. Using the phosphonate **12** instead of **11** gave the lactol **24** in a modest 33% yield, indicating that the

Table 1. Synthesis of thiomethylphosphono-*exo*-glycals **29**–**36**.



Entry	Lactone	Addition			Elimination			
		Lactol	*T* [h]	Yield [%][a]	*exo*-Glycal	*T* [h]	Yield [%][a]	Ratio (*Z*/*E*)[b]
1	**14**	**21**	1	99	**29**	2	80	70:30
2	**15**	**22**	3	56	**30**	5	87	60:40
3	**16**	**23**	1	88	**31**	2	55	92:8
4	**15**	**24**	22	33	**32**	4	60	70:30
5	**17**	**25**	2	77	**33**	2	99	96:4
6	**18**	**26**	3	42	**34**	3.5	75	40:60
7	**19**	**27**	2.5	71	**35**	5	48	65:35
8	**20**	**28**	5	51	**36**	5	51	70:30

[a] Isolated yield. [b] Determined by ^{31}P NMR spectroscopic analysis of the crude reaction mixture.

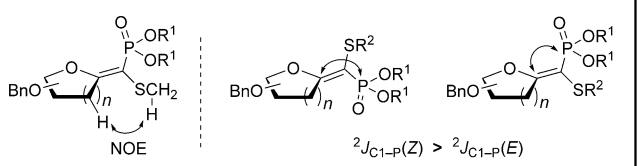
kinetics of the addition reaction of lithiated **12** to lactones is significantly slower than the methyl derivative **11**. This is probably because of the significant steric hindrance arising from the phenyl groups (Table 1, Entries 2–4). For all of the employed furanosides **14**–**17**, the formation of the (*Z*) isomer predominates and the (*Z*/*E*) ratios ranged from 60:40 to 96:4 (Table 1, Entries 1–5).

However, a moderate (*Z*) selectivity was observed for pyranosidic lactones in the reaction between **11** and the glucopyranoside **19** (Table 1, Entry 7). Surprisingly, the reaction with the galactopyranoside **18** gave preferentially the (*E*) isomer (Table 1, Entry 6). This difference in diastereoselectivity can be explained by the fact that the two pyranosides adopt different conformations in the transition state, because of the benzyl group at the C-4 position that is axial for **34** and equatorial for **35**. The result reported in Entry 5 (Table 1) show that protecting groups influence the course of the reaction. Indeed, a good yield of the elimination step (99%) with an excellent selectivity (*Z*/*E* 96:4) was observed when acetonides were used as protecting groups instead of benzyl ethers.

The *Z*/*E* assignment of tetrasubstituted *exo*-glycals is challenging, because the exocyclic double bond of **29**–**36** is substituted by four distinct elements (O, C, S, and P). However, their structures have been fully ascertained by NMR spectroscopy, using ^{1}H, ^{13}C, ^{31}P, $^{1}H/^{1}H$ correlations, and $^{1}H/^{13}C$ correlations (HSQC, HMBC). To demonstrate the configurations of all *exo*-glycals reported in this study, 1D homonuclear NOE spectra (1D NOE) were also acquired and compared for each *E*/*Z* diastereomeric pair.

For all (*E*)-configured enol ethers, 1D NOE experiments showed a NOE effect between the protons of the SCH_3 group and the H-2 proton of the carbohydrate ring, while the absence of this effect in their corresponding (*Z*) isomers unambiguously confirms the *Z*/*E* assignment (Table 2). Moreover, for all compounds **29**–**36**, the H-2 signal of the (*Z*) isomers are always shifted to lower field compared to the (*E*) isomers. The H-2 chemical shifts were recorded at 6.3–5.4 ppm for the (*Z*) isomers and 5.5–4.9 ppm for the (*E*) isomers. A similar trend was observed for the ^{31}P-signal chemical shifts, which were recorded between 19.7–17.8 ppm for the (*Z*) isomers and between 17.7–16.4 ppm for the (*E*) isomers. In addition, the coupling constants between C-1 and the phosphorous atom were always between 38–42 Hz when these two elements are *trans*, while this coupling constant was always close to 10 Hz when the ^{13}C and the ^{31}P are *cis* (Table 2).

Table 2. Comparison between $^{2}J_{C1-P}$ of both *exo*-glycals (*E*) and (*Z*).



Entry	*exo*-Glycal	$^{2}J_{C1-P}$ (*Z*)	$^{2}J_{C1-P}$ (*E*)
1	**29**	38.3	9.6
2	**30**	38.3	8.6
3	**31**	39.3	9.6
4	**32**	39.3	8.6
5	**33**	37.4	–
6	**34**	41.2	6.7
7	**35**	41.2	8.6
8	**36**	40.3	7.7

The *Z*/*E* diastereomers were very easily separated by standard silica gel chromatography except for compound **33**, which required a more tedious purification. We also observed that the (*Z*)-*exo*-glycals were always more polar than their (*E*) isomers (based on silica gel migratory ability). The latter trend is in agreement with all *cis*/*trans* phosphono-*exo*-glycals already described in our previous studies.[13a,25]

After optimizations, we found that the oxidation of the thiomethylphosphono-*exo*-glycal **29-(*Z*)** could be carried out using two equivalents of *m*-chloroperoxybenzoic acid (*m*-CPBA) in dichloromethane at 0 °C. After one hour, the methyl sulfide was chemoselectively oxidized leading to the desired product **37-(*Z*)** in quantitative yield (Table 3, Entry 1). Under these oxidizing conditions, the epoxidation of the enol ether was not observed. The second isomer **29-(*E*)** underwent the oxidation under the same conditions to afford **37-(*E*)** in 66% yield (Table 3, Entry 2). Changing the phosphonate alkyl group did not affect the course of the reaction, as the oxidation of both isomers **30-(*Z*)** and **30-(*E*)** provided the desired compounds **38-(*Z*)** and **38-(*E*)** in 68% and 69% yield, respectively (Table 3, Entries 3 and 4). In addition, a Me or Ph substituent on the sulphur atom had no significant effect in this oxidation (Table 3, Entries 3-6). The oxidation of **33-(*Z*)** was performed in 90% yield after only one hour, which indicates that an acetonide could be also used as a protecting group of the glycoside (Table 1, Entry 8). In the pyranose series, all the reactions starting from D-Gal, D-Glu, and L-Fuc (**34**–**36**) efficiently produced the corresponding sulfones **42**–**44** in good to excellent yields in a few hours (Table 2, Entries 9–12).

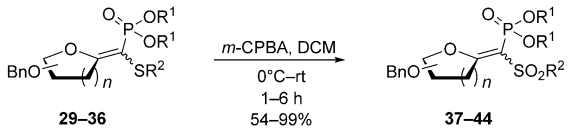
Globally, we were able to optimize a general methodology starting from a variety of lactones from the furanose and pyranose series. After the lithiated-phosphonate addition, the subsequent dehydration generally leads to the generation of the (*Z*) isomer.

The oxidation of all the resulting thioether *exo*-glycals chemoselectively gave the corresponding sulfones in good yields. Importantly, to the best of our knowledge, there are no other general synthetic methods yielding phosphonylated *exo*-glycals bearing two electron-withdrawing groups.

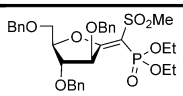
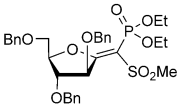
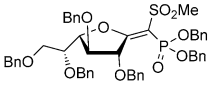
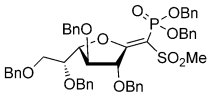
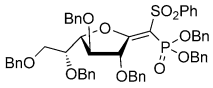
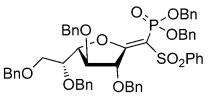
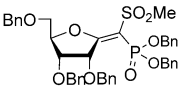
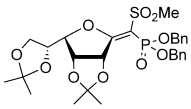
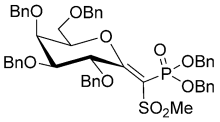
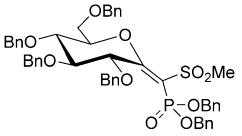
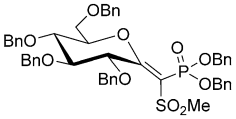
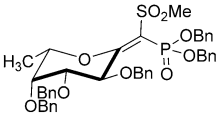
Given the importance of galactofuranose (Gal*f*) in the cell walls of major pathogens, we naturally explored the synthesis of modified phosphonylated analogs of this carbohydrate starting from **38-(*E*,*Z*)** (Scheme 2). To remove the benzyl groups, the usual hydrogenation conditions cannot be used because of the possibility of an enol ether saturation.[26] The deprotections were, thus, carried out with boron trichloride (BCl_3) in dry dichloromethane.[27] After two hours at temperatures between −78 °C and room temperature, the deprotected *exo*-glycals **45-(*Z*)** and **45-(*E*)** were quantitatively isolated for both isomers **38-(*Z*)** and **38-(*E*)**, respectively.

The coupling between **45-(*Z*)** and uridine 5′-phosphorimidazolide in DMF in the presence of $MgCl_2$ as the Lewis acid cata-

Table 3. Chemoselective oxidation of phosphono-*exo*-glycals **29–36**.

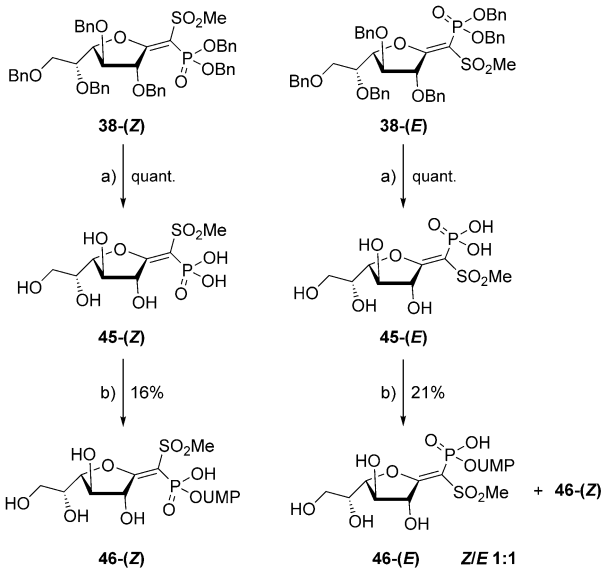


29–36 **37–44**

Entry	SM[a]	Structure	*T* [h]	Product	Yield[b] [%]
1	**29-(*Z*)**		1	**37-(*Z*)**	99
2	**29-(*E*)**		2	**37-(*E*)**	66
3	**30-(*Z*)**		1	**38-(*Z*)**	68
4	**30-(*E*)**		3	**38-(*E*)**	69
5	**32-(*Z*)**		6	**39-(*Z*)**	54
6	**32-(*E*)**		3	**39-(*E*)**	80
7	**31-(*Z*)**		3	**40-(*Z*)**	78
8	**33-(*Z*)**		1	**41-(*Z*)**	90
9	**34-(*E*)**		2	**42-(*E*)**	70
10	**35-(*Z*)**		4	**43-(*Z*)**	99
11	**35-(*E*)**		3	**43-(*E*)**	71
12	**36-(*Z*)**		2	**44-(*Z*)**	88

[a] SM = starting material. [b] Isolated yield of tetrasubstituted *exo*-glycals.

lyst afforded the desired unprotected nucleotide sugar **46-(*Z*)** in modest 16% yield.[28] To our surprise, the same coupling procedure starting from the other isomer **45-(*E*)** gave an *E*/*Z* mixture of the nucleotide sugar, which could be easily separated by size-exclusion chromatography and semi-preparative



Scheme 2. Reagents and conditions: a) BCl_3, DCM, −78 °C–rt, 2 h; b) Uridine 5′-phosphorimidazolide, $MgCl_2$, DMF, rt, 16 h.

HPLC purifications (Scheme 2). This result indicates that the (*E*) isomer is prone to an *E*-to-*Z* isomerization during the coupling step, whereas the *Z* isomer was configurationally stable.

Biochemical investigation

The two final molecules, **46-(*E*)** and **46-(*Z*),** were first assayed against UDP-galactopyranose mutase (UGM_{Mt} from *M. tuberculosis*) following two different procedures. First, the K_d values were determined using a well-established fluorescence-polarization assay.[29] This assay measures the competition between the inhibitors and a fluorescent probe for the nonreduced UGM (UDP-fluorescein) at a constant probe concentration and at varying concentrations of the inhibitors. We found that the (*E*) isomer **46-(*E*)** was a significantly better inhibitor (K_d = 77 μM) than its (*Z*) isomer (K_d = 470 μM). In our previous studies[13a,30] we had shown that *exo*-glycals could behave as time-dependent inactivators of *E. coli* UGM. In these experiments the (*E*) fluorinated isomer was found to be kinetically superior to its (*Z*) isomer; however, the relative affinity of both isomers towards UGM had not been measured. The K_d measurements of the sulfonylated exoglycals **46** clearly prove that UGM has a clear preference for the (*E*) isomer. It should also be emphasized that such an inhibition level against UGM (< 100 μM) has rarely been reached for UDP-galactose analogues in the literature.[2a,6c]

We then evaluated the ability of **46-(*Z*)** and **46-(*E*)** to behave as covalent irreversible inhibitors. Indeed, vinyl sulfones are generally excellent Michael acceptors, which often act as a covalent trap of enzyme nucleophilic residues.[15b,c] Moreover, the UGM reaction proceeds by a unique mechanism involving the cofactor as covalent nucleophile. The X-ray structure of UGM in complex with our fluorinated UDP-Gal*f* analogues confirms the spatial proximity of the FAD nucleophilic center and the Gal*f* anomeric position.[11d] Therefore, we measured the per-

centage of inhibition of the *exo*-glycals **46** by a standard HPLC activity assay (at UDP-Gal*f* 25 μM and inhibitor 500 μM). In these experiments the formation of UDP-Gal*p* is followed by HPLC at different reaction times. However, for each experiment, the enzyme and the inhibitor are pre-incubated before the addition of the substrate (UDP-Gal*f*), which starts the reaction. The pre-incubation time varies from one experiment to the other; if a covalent irreversible inhibition occurs, the percentage of inhibition should increase with pre-incubation time. Interestingly, independent of the pre-incubation time (0–30 min), the level of inhibition was always the same (23% and 46% for the (*Z*) and the (*E*) isomers, respectively, Table 4). Thus, sulfonylated *exo*-glycals are not time-dependent inactivators of UGM despite their Michael acceptor character. When compared to our preliminary inactivation studies using nonfluorinated and fluorinated *exo*-glycals,[13a,30] these results suggest that the enol ethers need to be protonated first to act as inactivators. This would generate an extremely electrophilic oxacarbenium ion that could react with the nucleophilic FAD cofactor. The strong electron-withdrawing character of the sulfone group on **46** likely decreases the propensity of the enol ether to be protonated. Therefore, a standard Michael addition (nucleophilic addition followed by a protonation) of the FAD cofactor on the electrophilic anomeric carbon of *exo*-glycals **46** does not occur. In addition, these experiments also gave us an estimation of the nucleophilicity of the FAD cofactor; if an electrophilic, non-reversible inhibitor were to be designed, it should possess an even more electrophilic functional group than the sulfonylated enol ether present in molecules **46**.

We then pursued the biochemical investigation with the two subsequent mycobacterial Gal*f* processing enzymes, the galactofuranosyltransferases Gl*f*T1 and Gl*f*T2. The Gl*f*T1 and Gl*f*T2 inhibition assays are based on the same principle; in both cases the inhibitors compete with the donor substrate for the transferase UDP-Gal*f* (**2**) in presence of a specific acceptor substrate (glycosyl phospholipid **47** and trisaccharide **48** for Gl*f*T1 and Gl*f*T2, respectively; see the Supporting Information for detailed procedures and Figure 3 for the structures). In both cases, the production of UDP during the glycosyl transfer reaction is coupled with a NADH oxidation, which can be easily monitored spectrophotometrically.[12f]

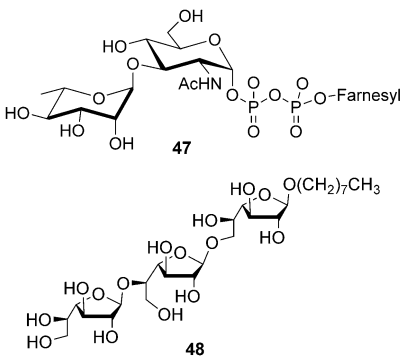


Figure 3. Structures of acceptor substrates **47** and **48** employed for the Gl*f*T1 and Gl*f*T2 assays, respectively.

Interestingly, the (*E*) isomer **46-(*E*)** showed a better affinity for Gl*f*T1 than its (*Z*) isomer (Table 4), while **46-(*Z*)** proved to be superior for Gl*f*T2. The level of inhibition is in the range of the affinity of the donor substrate **2** for the two transferases

Table 4. Inhibition of UGM, Gl*f*T1, and Gl*f*T2 by **46-(*Z*)** and **46-(*E*)**.

	UGM_{Mt}		Gl*f*T1		Gl*f*T2	
	K_d[a] [μM]	Inhib[b] [%]	IC_{50}[c] [mM]	Inhib[d] [%]	IC_{50}[e] [mM]	Inhib[f] [%]
46-(*Z*)	470 ± 2	23 ± 1	–[g]	33 ± 6	3.85 ± 0.06	55 ± 4
46-(*E*)	77 ± 1	46 ± 1	1.09 ± 0.02	62 ± 2	–[g]	26 ± 5

[a] FP assay: fluorescent probe (15 nM), UGM (580 nM). [b] HPLC assay: *I* (Inhibitor, 500 μM), reduced UGM (60 nM), UDP-Gal*f* (25 μM). [c] *I* (range, see the Supporting Information for details), UDP-Gal*f* (3 mM), acceptor (2 mM). [d] *I* (4 mM), UDP-Gal*f* (3 mM), acceptor (2 mM). [e] *I* (range, see the Supporting Information for details), UDP-Gal*f* (3 mM), acceptor (2 mM). [f] *I* (4 mM), UDP-Gal*f* (3 mM), acceptor (2 mM). [g] IC_{50} values were determined only for compounds displaying >50% inhibition at *I* = 4 mM.

(K_m(Gl*f*T1) = 566 μM;[31] K_m(Gl*f*T2) = 380 μM[12f]). These results are consistent with the inhibition data measured for glycosyltransferases in general. Indeed, the very best nucleotide-sugar analogues reported as glycosyltransferase inhibitors usually display inhibition levels at concentrations around the K_m of the enzyme-donor substrate.[14,32] This study is the first example in which the relative affinities of the same donor analogues are measured against both *M. tuberculosis* galactofuranosyltransferases. Perhaps most interesting, these experiments show that the two enzymes do not accommodate UDP-Gal*f* the same way, because they show a distinct binding preference for the two *Z*/*E* diastereomers.

While Gl*f*T1 has no well characterized inhibitor to date, several attempts have been made to inhibit Gl*f*T2; Gal*f*,[12a,33] UDP,[12e] UDP-Gal*f*,[12b,c] and acceptor analogues[12d] have been produced and assayed against this transferase. The inhibition level usually observed is in the same range like that seen for **46**, which leaves open the question of how to design tight-binding Gl*f*T inhibitors.

The 3D structures of Gl*f*T2, free and in complex with UDP, have been recently obtained by X-ray crystallography.[34] These structures revealed a unique tetrameric topology that shed light on the mechanism of the galactan polymerization, a central and debated question.[9c,35] In absence of X-ray structures of both Gl*f*T1 and Gl*f*T2 in complex with UDP-Gal*f* (or at least UDP), the experiments detailed in this study provide useful information about the relative binding modes of the nucleotide sugars within the catalytic pockets of both transferases.

Conclusion

This study reports a novel methodology to synthesize *exo*-glycals bearing two electron-withdrawing groups, a sulfone and a phosphonate. This methodology was generalized to eight different carbohydrates from the furanose and pyranose series. The *Z*/*E* configurations of these tetrasubstituted enol ethers

could be ascertained using NMR spectroscopic techniques. Deprotection of the *exo*-glycals followed by an UMP coupling generated two original UDP-Gal*f* analogues **46-(*E*)** and **46-(*Z*)**. The *Z/E* isomers were independently evaluated as inhibitors of UGM, Gl*f*T1, and Gl*f*T2, the three mycobacterial galactofuranose processing enzymes. Molecule **46-(*E*)** was, thus, the first characterized inhibitor of Gl*f*T1 reported to date and was also found to efficiently inhibit UGM in a reversible manner. Interestingly, Gl*f*T2 showed a better affinity for **46-(*Z*).**

This study nicely complements the pioneering work of Ferrières, Mikusova, and Daniellou who compared a series of synthetic UDP-furanoses on cell-free assays, in which both Gl*f*T1 and Gl*f*T2 were present.[3d] Although this earlier study did not report the relative affinities of these nucleotide sugars towards the enzymes, it showed that some of the molecules were substrates of Gl*f*T1. These compounds were found to inhibit the galactan polymerization by producing short "dead-end" intermediates, resulting from the incorporation of the modified carbohydrate residues into the growing carbohydrate chain.

The three enzymes studied in the present work are not only interesting because, mechanistically, they are still the topic of intense investigations, but also because they constitute very important targets for the development of novel antimycobacterial agents. A very recent study showed that Gl*f*T2 plays a key role in the spatiotemporal cell growth and cell-envelope elongation in mycobacteria,[36] thus reinforcing the interest in the fundamental understanding of this enzyme.

Experimental Section

All the experimental details are gathered in the Supporting Information.

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Keywords: glycals · glycosyltransferase · phosphonates · sulfones · tuberculosis

[1] a) S. M. Blower, T. Chou, *Nat. Med.* **2004**, *10*, 1111 – 1116; b) A. Koul, E. Arnoult, N. Lounis, J. Guillemont, K. Andries, *Nature* **2011**, *469*, 483 – 490.

[2] a) G. Eppe, S. E. Bkassiny, S. P. Vincent, *Galactofuranose Biosynthesis: Discovery, Mechanisms and Therapeutic Relevance*, The Royal Society of Chemistry, Cambridge, **2015**, pp. 209 – 241; b) I. Chlubnova, L. Legentil, R. Dureau, A. Pennec, M. Almendros, R. Daniellou, C. Nugier-Chauvin, V. Ferrières, *Carbohydr. Res.* **2012**, *356*, 44 – 61.

[3] a) K. Mikusova, T. Yagi, R. Stern, M. R. McNeil, G. S. Besra, D. C. Crick, P. J. Brennan, *J. Biol. Chem.* **2000**, *275*, 33890 – 33897; b) L. Kremer, L. G. Dover, C. Morehouse, P. Hitchin, M. Everett, H. R. Morris, A. Dell, P. J. Brennan, M. R. McNeil, C. Flaherty, K. Duncan, G. S. Besra, *J. Biol. Chem.* **2001**, *276*, 26430 – 26440; c) D. C. Crick, S. Mahapatra, P. J. Brennan, *Glycobiology* **2001**, *11*, 107R – 118R; d) P. Peltier, M. Belanova, P. Dianiskova, R. Zhou, R. B. Zheng, J. A. Pearcey, M. Joe, P. J. Brennan, C. Nugier-Chauvin, V. Ferrieres, T. L. Lowary, R. Daniellou, K. Mikusova, *Chem. Biol.* **2010**, *17*, 1356 – 1366.

[4] S. K. Angala, J. M. Belardinelli, E. Huc-Claustre, W. H. Wheat, M. Jackson, *Crit. Rev. Biochem. Mol. Biol.* **2014**, *49*, 361 – 399.

[5] a) P. M. Nassau, S. L. Martin, R. E. Brown, A. Weston, D. Monsey, M. R. McNeil, K. Duncan, *J. Bacteriol.* **1996**, *178*, 1047 – 1052; b) J. N. Barlow, M. E. Girvin, J. S. Blanchard, *J. Am. Chem. Soc.* **1999**, *121*, 6968 – 6969; c) Q. Zhang, H.-w. Liu, *J. Am. Chem. Soc.* **2000**, *122*, 9065 – 9070; d) S. Bornemann, *Nat. Prod. Rep.* **2002**, *19*, 761 – 772; e) S. O. Mansoorabadi, C. J. Thibodeaux, H.-w. Liu, *J. Org. Chem.* **2007**, *72*, 6329 – 6342; f) M. Soltero-Higgin, E. E. Carlson, T. D. Gruber, L. L. Kiessling, *Nat. Struct. Mol. Biol.* **2004**, *11*, 539 – 543.

[6] a) S. Berg, D. Kaur, M. Jackson, P. J. Brennan, *Glycobiology* **2007**, *17*, 35R – 56R; b) P.-H. Tam, T. L. Lowary, *Curr. Opin. Chem. Biol.* **2009**, *13*, 618 – 625; c) M. R. Richards, T. L. Lowary, *ChemBioChem* **2009**, *10*, 1920 – 1938.

[7] a) L. J. Alderwick, L. G. Dover, N. Veerapen, S. S. Gurcha, L. Kremer, D. L. Roper, A. K. Pathak, R. C. Reynolds, G. S. Besra, *Protein Expression Purif.* **2008**, *58*, 332 – 341; b) M. A. Martinez Farias, V. A. Kincaid, V. R. Annamalai, L. L. Kiessling, *J. Am. Chem. Soc.* **2014**, *136*, 8492 – 8495.

[8] N. L. Rose, G. C. Completo, S.-J. Lin, M. McNeil, M. M. Palcic, T. L. Lowary, *J. Am. Chem. Soc.* **2006**, *128*, 6721 – 6729.

[9] a) J. F. May, R. A. Splain, C. Brotschi, L. L. Kiessling, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 11851 – 11856; b) A. Bernardi, J. Jiménez-Barbero, A. Casnati, C. De Castro, T. Darbre, F. Fieschi, J. Finne, H. Funken, K.-E. Jaeger, M. Lahmann, T. K. Lindhorst, M. Marradi, P. Messner, A. Molinaro, P. V. Murphy, C. Nativi, S. Oscarson, S. Penades, F. Peri, R. J. Pieters, O. Renaudet, J.-L. Reymond, B. Richichi, J. Rojo, F. Sansone, C. Schaeffer, W. B. Turnbull, T. Velasco-Torrijos, S. Vidal, S. Vincent, T. Wennekes, H. Zuilhof, A. Imberty, *Chem. Soc. Rev.* **2013**, *42*, 4709 – 4727; c) M. R. Levengood, R. A. Splain, L. L. Kiessling, *J. Am. Chem. Soc.* **2011**, *133*, 12758 – 12766.

[10] a) G. C. Completo, T. L. Lowary, *J. Org. Chem.* **2008**, *73*, 4513 – 4525; b) M. G. Szczepina, R. B. Zheng, G. C. Completo, T. L. Lowary, B. M. Pinto, *ChemBioChem* **2009**, *10*, 2052 – 2059; c) M. G. Szczepina, R. B. Zheng, G. C. Completo, T. L. Lowary, B. M. Pinto, *Bioorg. Med. Chem.* **2010**, *18*, 5123 – 5128.

[11] a) C. Ansiaux, I. N'go, S. P. Vincent, *Chem. Eur. J.* **2012**, *18*, 14860 – 14866; b) S. El Bkassiny, I. N'Go, C. M. Sevrain, A. Tikad, S. P. Vincent, *Org. Lett.* **2014**, *16*, 2462 – 2465; c) I. N'Go, S. Golten, A. Ardá, J. Cañada, J. Jiménez-Barbero, B. Linclau, S. P. Vincent, *Chem. Eur. J.* **2014**, *20*, 106 – 112; d) K. E. van Straaten, C. M. Sevrain, S. A. Villaume, J. Jiménez-Barbero, B. Linclau, S. P. Vincent, D. A. R. Sanders, *J. Am. Chem. Soc.* **2015**, *137*, 1230 – 1244; e) A. Caravano, S. P. Vincent, *Eur. J. Org. Chem.* **2009**, 1771 – 1780; f) G. Eppe, P. Peltier, R. Daniellou, C. Nugier-Chauvin, V. Ferrières, S. P. Vincent, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 814 – 816; g) A. Caravano, P. Sinaÿ, S. P. Vincent, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1123 – 1125.

[12] a) J. Li, T. L. Lowary, *Med. Chem. Commun.* **2014**, *5*, 1130 – 1137; b) M. B. Poulin, R. Zhou, T. L. Lowary, *Org. Biomol. Chem.* **2012**, *10*, 4074 – 4087; c) K. Vembaiyan, J. A. Pearcey, M. Bhasin, T. L. Lowary, W. Zou, *Bioorg. Med. Chem.* **2011**, *19*, 58 – 66; d) J. Frigell, J. A. Pearcey, T. L. Lowary, I. Cumpstey, *Eur. J. Org. Chem.* **2011**, 1367 – 1375; e) A. E. Trunkfield, S. S. Gurcha, G. S. Besra, T. D. Bugg, *Bioorg. Med. Chem.* **2010**, *18*, 2651 – 2663; f) N. L. Rose, R. B. Zheng, J. Pearcey, R. Zhou, G. C. Completo, T. L. Lowary, *Carbohydr. Res.* **2008**, *343*, 2130 – 2139.

[13] a) A. Caravano, H. Dohi, P. Sinaÿ, S. P. Vincent, *Chem. Eur. J.* **2006**, *12*, 3114 – 3123; b) L. Dumitrescu, G. Eppe, A. Tikad, W. Pan, S. E. Bkassiny, S. S. Gurcha, A. Ardá, J. Jiménez-Barbero, G. S. Besra, S. P. Vincent, *Chem. Eur. J.* **2014**, *20*, 15208 – 15215.

[14] a) M. D. Burkart, S. P. Vincent, A. Düffels, B. W. Murray, S. V. Ley, C.-H. Wong, *Bioorg. Med. Chem.* **2000**, *8*, 1937 – 1946; b) L. Tedaldi, G. K. Wagner, *Med. Chem. Commun.* **2014**, *5*, 1106 – 1125.

[15] a) J. J. Reddick, J. Cheng, W. R. Roush, *Org. Lett.* **2003**, *5*, 1967 – 1970; b) L. S. Brinen, E. Hansell, J. Cheng, W. R. Roush, J. H. McKerrow, R. J. Fletterick, *Structure* **2000**, *8*, 831 – 840; c) E. Dunny, P. Evans, *Curr. Bioact. Compd* **2011**, *7*, 218 – 236.

[16] a) M. Lakhrissi, Y. Chapleur, *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 750 – 752; *Angew. Chem.* **1996**, *108*, 833 – 834; b) Y. Lakhrissi, C. Taillefumier, M. Lakhrissi, Y. Chapleur, *Tetrahedron: Asymmetry* **2000**, *11*, 417 – 421; c) Y. Lakhrissi, C. Taillefumier, F. Chretien, Y. Chapleur, *Tetrahedron Lett.* **2001**, *42*, 7265 – 7268.

[17] a) F. K. Griffin, D. E. Paterson, P. V. Murphy, R. J. K. Taylor, *Eur. J. Org. Chem.* **2002**, 1305 – 1322; b) Y. Ohnishi, Y. Ichikawa, *Bioorg. Med. Chem. Lett.* **2002**, *12*, 997 – 999; c) G. D. McAllister, D. E. Paterson, R. J. K. Taylor, *Angew. Chem. Int. Ed.* **2003**, *42*, 1387 – 1391; *Angew. Chem.* **2003**, *115*, 1425 – 1429; d) R. J. K. Taylor, G. D. McAllister, R. W. Franck, *Carbohydr. Res.* **2006**, *341*, 1298 – 1311; e) P. Pasetto, R. W. Franck, *J. Org. Chem.* **2003**, *68*, 8042 – 8060.

[18] a) M. Benltifa, S. Vidal, D. Gueyrard, P. G. Goekjian, M. Msaddek, J.-P. Praly, *Tetrahedron Lett.* **2006**, *47*, 6143 – 6147; b) B. Bourdon, M. Corbet, P. Fontaine, P. G. Goekjian, D. Gueyrard, *Tetrahedron Lett.* **2008**, *49*, 747 – 749; c) D. Gueyrard, R. Haddoub, A. Salem, N. S. Bacar, P. G. Goekjian, *Synlett* **2005**, 520 – 522; d) D. Gueyrard, P. Fontaine, P. G. Goekjian, *Synthesis* **2006**, 1499 – 1503.

[19] a) W. B. Yang, C. F. Chang, S. H. Wang, C. F. Teo, C. H. Lin, *Tetrahedron Lett.* **2001**, *42*, 4657 – 4660; b) W. B. Yang, C. Y. Wu, C. C. Chang, S. H. Wang, C. F. Teo, C. H. Lin, *Tetrahedron Lett.* **2001**, *42*, 6907 – 6910; c) W.-B. Yang, Y.-Y. Yang, Y.-F. Gu, S.-H. Wang, C.-C. Chang, C.-H. Lin, *J. Org. Chem.* **2002**, *67*, 3773 – 3782.

[20] R. N. Monrad, R. Madsen, *Tetrahedron* **2011**, *67*, 8825 – 8850.

[21] a) A. Gómez, A. Pedregosa, A. Barrio, S. Valverde, C. Lopez, *Tetrahedron Lett.* **2004**, *45*, 6307 – 6310; b) A. M. Gómez, A. Barrio, A. Pedregosa, C. Uriel, S. Valverde, J. C. Lopez, *Eur. J. Org. Chem.* **2010**, 2910 – 2920.

[22] a) A. Novoa, N. Pellegrini-Moise, S. Lamande-Langle, Y. Chapleur, *Tetrahedron Lett.* **2009**, *50*, 6484 – 6487; b) A. M. Gómez, G. O. Danelon, A. Pedregosa, S. Valverde, J. C. Lopez, *Chem. Commun.* **2002**, 2024 – 2025; c) A. Novoa, N. Pellegrini-Moise, S. Bourg, S. Thoret, J. Dubois, G. Aubert, T. Cresteil, Y. Chapleur, *Eur. J. Med. Chem.* **2011**, *46*, 3570 – 3580; d) H.-T. T. Thien, A. Novoa, N. Pellegrini-Moïse, F. Chrétien, C. Didierjean, Y. Chapleur, *Eur. J. Org. Chem.* **2011**, 6939 – 6951.

[23] A. M. Gómez, A. Barrio, I. Amurrio, S. Valverde, S. Jarosz, J. C. Lopez, *Tetrahedron Lett.* **2006**, *47*, 6243 – 6246.

[24] a) J.-S. Thomann, F. Monneaux, G. Creusat, M. V. Spanedda, B. Heurtault, C. Habermacher, F. Schuber, L. Bourel-Bonnet, B. Frisch, *Eur. J. Med. Chem.* **2012**, *51*, 174 – 183; b) L. M. Lerner, B. D. Kohn, P. Koh, *J. Org. Chem.* **1968**, *33*, 1780 – 1783; c) K. Bennis, P. Calinaud, J. Gelas, M. Ghobsi, *Carbohydr. Res.* **1994**, *264*, 33 – 44.

[25] G. Eppe, L. Dumitrescu, O. Pierrot, T. Li, W. Pan, S. P. Vincent, *Chem. Eur. J.* **2013**, *19*, 11547 – 11552.

[26] a) A. Caravano, W. Pan, S. P. Vincent, *Arkivoc* **2007**, *x*, 348 – 364; b) A. Caravano, D. Mengin-Lecreulx, J.-M. Brondello, S. P. Vincent, P. Sinaÿ, *Chem. Eur. J.* **2003**, *9*, 5888 – 5898.

[27] a) D. R. Williams, D. L. Brown, J. W. Benbow, *J. Am. Chem. Soc.* **1989**, *111*, 1923 – 1925; b) V. Liautard, V. Desvergnes, K. Itoh, H.-w. Liu, O. R. Martin, *J. Org. Chem.* **2008**, *73*, 3103 – 3115.

[28] a) T. Li, A. Tikad, W. Pan, S. P. Vincent, *Org. Lett.* **2014**, *16*, 5628 – 5631; b) P. Dabrowski-Tumanski, J. Kowalska, J. Jemielity, *Eur. J. Org. Chem.* **2013**, 2147 – 2154.

[29] M. Soltero-Higgin, E. E. Carlson, J. H. Phillips, L. L. Kiessling, *J. Am. Chem. Soc.* **2004**, *126*, 10532 – 10533.

[30] A. Caravano, S. P. Vincent, P. Sinaÿ, *Chem. Commun.* **2004**, 1216 – 1217.

[31] A. Koizumi, R. B. Zhang, T. L. Lowary, unpublished results.

[32] a) S. Wang, S. Vidal, *Carbohydr. Chem.* **2013**, *39*, 78 – 101; b) H. Dohi, R. Périon, M. Durka, M. Bosco, Y. Roué, F. Moreau, S. Grizot, A. Ducruix, S. Escaich, S. P. Vincent, *Chem. Eur. J.* **2008**, *14*, 9530 – 9539; c) M. D. Burkart, S. P. Vincent, C.-H. Wong, *Chem. Commun.* **1999**, 1525 – 1526.

[33] S. Cren, A. J. Blake, G. S. Besra, N. R. Thomas, S. S. Gurcha, *Org. Biomol. Chem.* **2004**, *2*, 2418 – 2420.

[34] R. W. Wheatley, R. B. Zheng, M. R. Richards, T. L. Lowary, K. K. S. Ng, *J. Biol. Chem.* **2012**, *287*, 28132 – 28143.

[35] J. F. May, M. R. Levengood, R. A. Splain, C. D. Brown, L. L. Kiessling, *Biochemistry* **2012**, *51*, 1148 – 1159.

[36] J. M. Hayashi, C.-Y. Luo, J. A. Mayfield, T. Hsu, T. Fukuda, A. L. Walfield, S. R. Giffen, J. D. Leszyk, C. E. Baer, O. T. Bennion, A. Madduri, S. A. Shaffer, B. B. Aldridg, C. M. Sassetti, S. J. Sandler, T. Kinoshit, D. B. Moody, Y. S. Morita, *Proc. Natl. Acad. Sci. USA* **2016**, *113*, 5400 – 5405.

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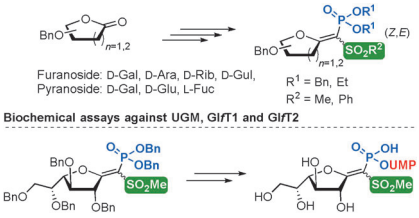
FULL PAPER

New antimycobacterial agents: This study reports a new methodology to synthesize *exo*-glycals bearing both a sulfone and a phosphonate, which was applied to prepare two original UDP-galactofuranose analogues. The latter were evaluated as inhbitors of UGM, Glf*T*1, and Glf*T*2, three enzymes involved in the *Mycobacterium tuberculosis* cell wall biosynthesis.



Carbohydrates

*C. J.-M. Frédéric, A. Tikad, J. Fu, W. Pan, R. B. Zheng, A. Koizumi, X. Xue, T. L. Lowary, S. P. Vincent**

■■ – ■■

Synthesis of Unprecedented Sulfonylated Phosphono-*exo*-Glycals Designed as Inhibitors of the Three Mycobacterial Galactofuranose Processing Enzymes