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Triazole-Linked Iminosugars and Aromatic Systems as Simplified UDP-Gal*f* Mimics: Synthesis and Preliminary Evaluation as Gal*f*-transferase Inhibitors

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Abstract: The convenient preparation of 1,4-dideoxy-1,4-imino-L-arabinitol derivatives carrying a propargyl group by way of the addition of TMS-propargyl Grignard reagent to a *N*-sulfinylglycosylamine is reported. This compound was coupled via a triazole-tether to various aromatic and heteroaromatic groups, thus providing structures that can be considered as simplified mimics of UDP-Gal*f*, the substrate of GlfT2, a galactofuranosyltransferase present in mycobacteria. These new compounds were evaluated as inhibitors of this enzyme and found to have significant activity (IC_{50} values in the high μM to low mM range) in spite of the absence of the diphospho linkage present in the parent sugar nucleotide.

Introduction

1-*C*-substituted iminosugars are stable glycoside mimics having significant biological activities as inhibitors of enzymes acting on sugars.[1] This activity is commonly attributed to their structural features and to their resemblance to the oxocarbenium ion-like transition state that typically characterizes (i) enzymatic glycosidase hydrolysis and (ii) enzymatic glycosyl transfer involved in the formation of *N*, *S,* or *O*-glycoconjugates.[1-3]

Many iminosugar-1-*C*-glycosides are thus powerful inhibitors of glycosidases,[1,4] purine nucleoside phosphorylases, nucleoside hydrolases,[5] and also potential inhibitors of glycosyltransferases.[1,6] Some have found applications as pharmacological chaperones to treat deficiencies resulting from improperly folded proteins.[7]

As such, 1-*C*-propargyl iminosugars are highly desirable precursors for the synthesis of a diversity of imino-*C*-glycosyl compounds useful for biological labeling and for the preparation of multivalent iminosugar scaffolds. As shown by Compain et al, the latter may exhibit outstanding binding enhancements to proteins compared to monovalent ligands and have thus stimulated (bio)chemists[8] to exploit various bioorthogonal ligation processes, and especially the copper-catalyzed azide–alkyne cycloaddition (e.g., CuAAC or click chemistry) to circumvent the typical “lock and key”, single-molecule approach.

However, while several examples of *N*- and *O*-armed alkynyl-iminosugars have been published, a single example of a 1-*C*-propargyl compound has been reported so far in the piperidine series (i.e., 1-*C*-propargyl-1-deoxyimino-D-xylitol).[9] A few examples based on a pyrrolidine core have been obtained by organocatalysis[10] or through addition to cyclic *N*,*O*-acetals[11] or to cyclic nitrones.[12]

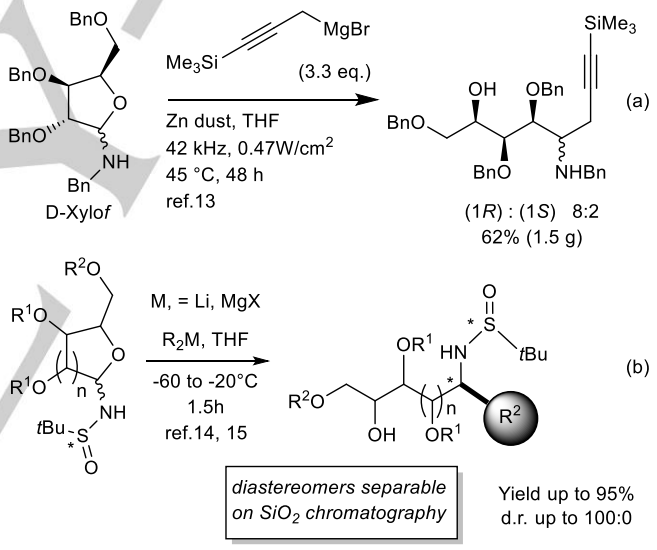

Figure 1. Previous work towards the synthesis of 1-*C*-iminosugars.

In our group, we have previously shown that the straightforward preparation of a 1-*C*-(2-propynyl)-1,4-imino-L-arabinitol derivative could be achieved by way of the addition of a propargyl zinc reagent to *N*-benzyl-D-xylofuranosylamine using ultrasound as a critical mode of activation (Figure 1, path a).[13] However, the yield and stereoselectivity of the reaction were moderate and the diastereomers were most often not separable using conventional techniques, even after successive post functionalization sequences.

In the meantime, we have demonstrated that *N*-*t*-butanesulfinyl glycosylamines were more reliable and versatile synthetic intermediates *en route* to imino-*C*-glycosyl compounds compared to their N-benzyl equivalents.[14,15] The methodology proceeds through the addition of magnesium or lithium reagents to *N*-*t*-butanesulfinyl glycosylamines to give, after further mesylation and treatment with a base, iminosugar-*C*-glycosides.

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The diastereoselectivity of the addition process could be boosted using LiCl as an additive,[14a] and in some cases controlled by the chiral sulfinyl group.[14b] The diastereomers, which were obtained in good yields and good stereoselectivities, were all separable through classical preparative chromatography columns (SiO_2) using either (S_R)-*N* or (S_S)-*N* or both related sulfinyl glycosylamines (Figure 1, path b).[14]

Considering the value of 1-*C*-propargyl iminosugar derivatives as reaction partners in a diversity of click reactions, our ongoing program on imino-*C*-glycosyl compouds was extended to triazole-linked imino-L-arabinitols as simplified uridinyl diphospho-D-galactofuranose (UDP-Gal*f*) mimics and possible inhibitors of GlfT2, a key galactofuranosyltransferase involved in the assembly of the cell wall of mycobacteria, including the causative agent of tuberculosis, *Mycobacterium tuberculosis*.[16] GlfT2 is responsible for adding the bulk of the galactofuranose residues into cell wall arabinogalactan (AG), the production of which is essential for the mycobacterial viability.[17] Clinically used drugs for treating tuberculosis target AG biosynthesis,[18] and it is believed that potent inhibitors of GlfT2 will have therapeutic potential. For this we decided to improve the synthesis of 1-*C*-(2-propynyl)-1,4-imino-L-arabinitols using our *N*-*t*-Bu-sulfinylamine methodology.[14]

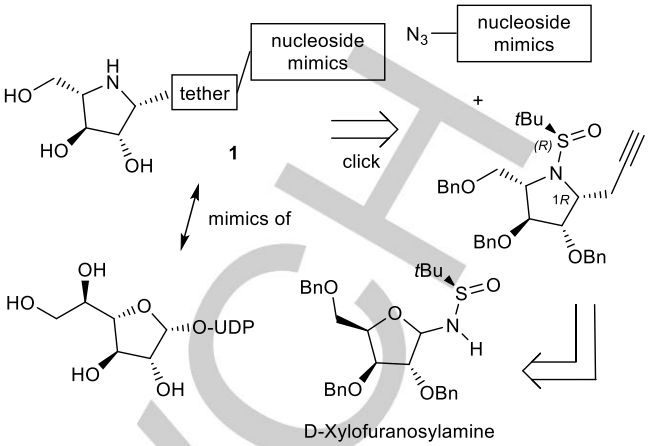


Figure 2. Retrosynthetic Planning.

We report herein the highly efficient synthesis of such compounds and their utilization for the preparation of a small library of pyrrolidine iminosugars conjugated to an aromatic or heteroaromatic moiety by a triazole tether (see compounds of type **1,** Figure 2). Preliminary studies of the biological activities of these compounds as GlfT2 inhibitors are also reported.

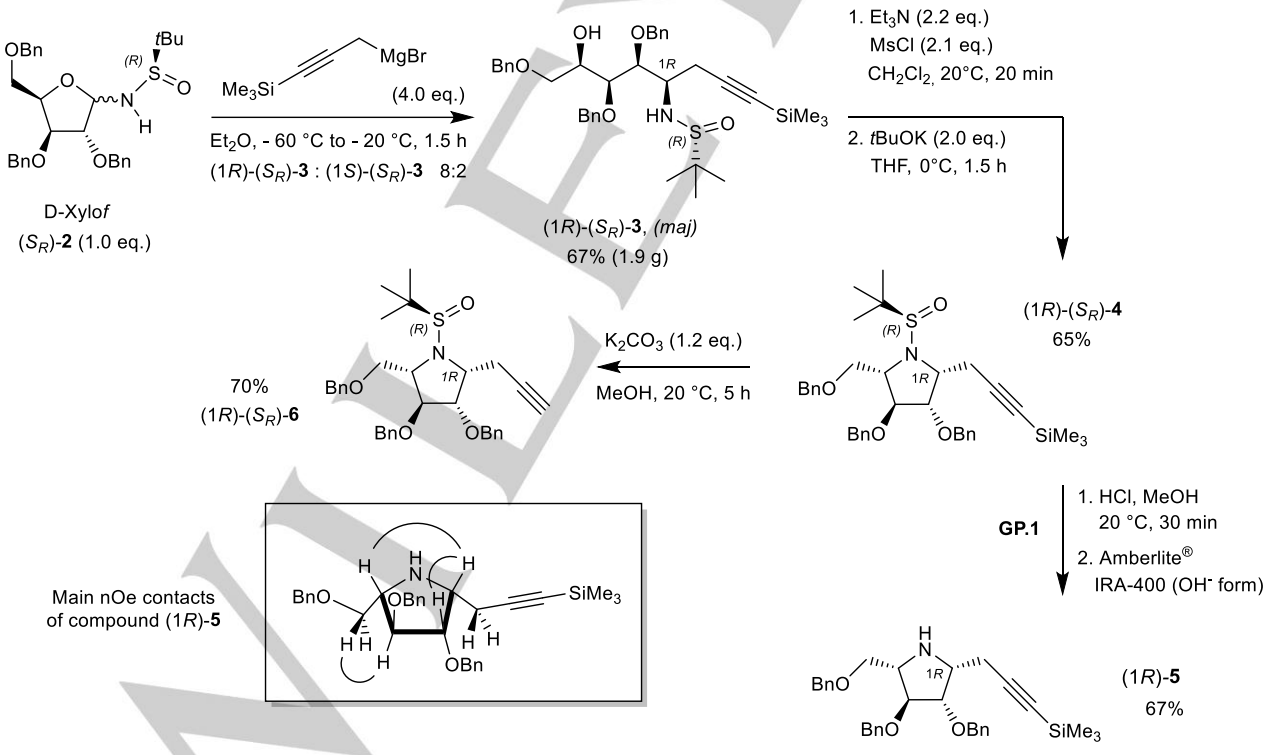


Figure 3. Synthesis of (1*R*)-(S_R)-**6** and determination of the configuration at C-1.

Results and Discussion

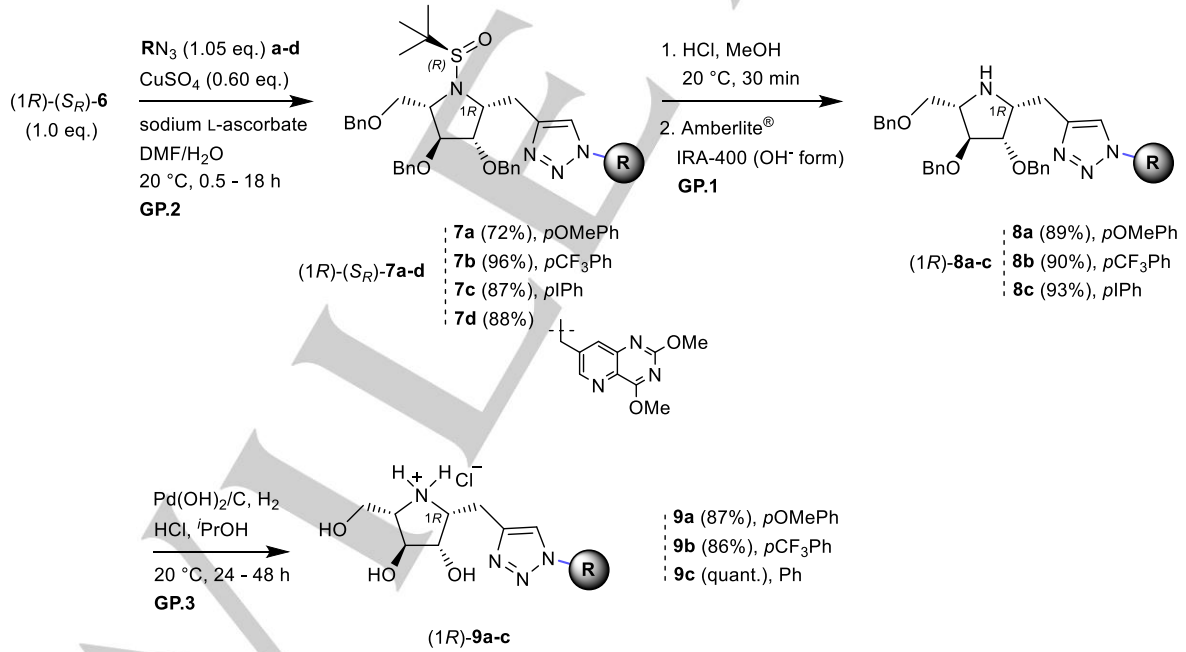
Synthesis

Retrosynthetic analysis of L-*arabino* 1-*C*-propargyl-iminopentitols (Gal*f* mimics) requires a D-*xylo*-furanosylamine as the starting material. The addition of a metallated propargylic species, followed by a cyclization with inversion at C-4, would generate the desired 1-(2-propynyl)-1,4-dideoxy-1,4-imino-L-arabinitol (Figure 2).[13]

(S$_R$*)-N-tert*-butanesulfinyl-D-xylofuranosylamine (*S*$_R$)-**2** (Figure 3) was thus generated in one step from 2,3,5-tri-*O*-benzyl-D-xylofuranose in the presence of $Ti(OEt)_4$ in toluene at 70 °C (71%).[15] Propargylation was then performed using a mercury-free protocol as previously employed by Kobayashi et al.[19] In this approach, 3-(trimethylsilyl)-2-propynyl magnesium bromide was generated using a catalytic amount of $ZnBr_2$ (8 mol%), magnesium turnings (3.4 equiv.) and trimethylsilylpropargyl bromide (1.0 equiv.) in Et_2O at 0 °C. Subsequent addition of this reagent to (*S*$_R$)-**2** at low temperature (–60 °C to –20 °C) gave (*S*$_R$)-**3** as a 8:2 mixture of diastereomers in 84% yield. To verify the stereoselectivity of the propargylation process, the major diastereomer of compound **3** was separated (SiO_2) and independently cyclized under typical conditions (MsCl, Et_3N in CH_2Cl_2, then *t*BuOK in THF) to provide 1-*C*-(3-trimethylsilyl-2-propynyl)-1,4-imino-L-arabinitol (*S*$_R$)-**4** in good yield (65%).

Removal of the chiral sulfinyl group was subsequently achieved using HCl in MeOH to give **5**. After neutralization, compound **5** was purified by column chromatography (67%, see general procedure 1, GP.1). ^{1}H NMR, ^{13}C NMR, correlation spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY) allowed determination of the configuration at C-1. The main nOe contacts of compound (1*R*)-**5** are shown in Figure 3. On the basis of correlations between H-1 and H-4, H-3 and H-5, compound (1*R*)-**5**, arising from 1,2-*cis*-(**3**) i.e., (1*R*)-(*S*$_R$)-**3**, was established to be the major diastereomer. This indicates possible stereocontrol via a Cram chelate and no participation of the sulfinyl auxiliary in the addition of simple Grignard reagents.[14a]

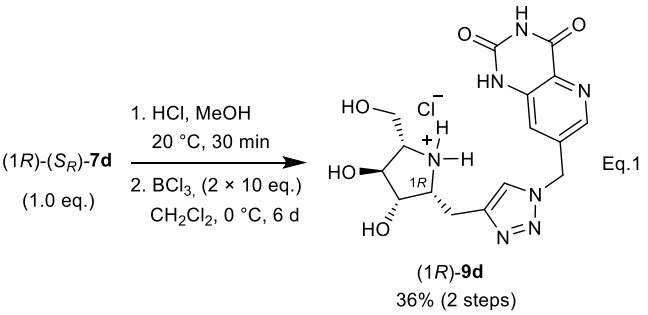
The trimethylsilyl protecting group of compound (1*R*)-(*S*$_R$)-**4** was then removed (K_2CO_3 in MeOH) to afford (1*R*)-(*S*$_R$)-**6** in 70% yield. From this building block, and a set of azido-compouds (i.e., compounds **a–d**, see ESI), simple UDP-Gal*f* analogues were then synthesized by CuAAC click chemistry (see GP.2, Scheme 1). This set of molecules was chosen to ensure a relatively high degree of diversity for future potential Gal*f* mimics. A 4-methoxyphenyl (series a), a 4-trifluoromethylphenyl (series b), a 4-iodophenyl (series c), and a 2,4-dimethoxypyrido[3,2-d]pyrimidine (series d) group were thus selected. The corresponding derivatives **7a–d** were obtained in fairly good yields (72–96%) by this cycloaddition process.



Scheme 1. Preparation of UDP-Gal*f* Mimics (1*R*)-**9a–c**.

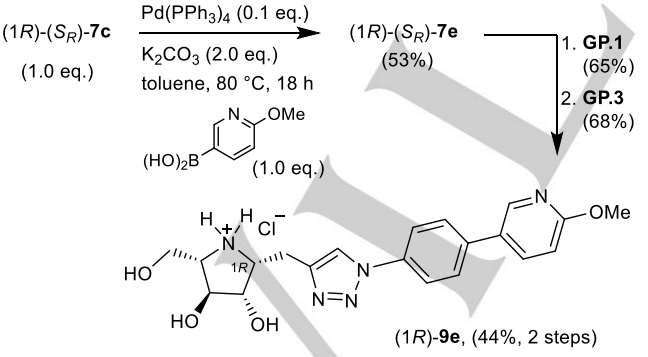
Next, the sulfinyl protecting group of compounds **7a–c** was cleaved following GP.1, to give (1*R*)-**8a–c** in excellent yields (89-93%). Hydrogenolysis using GP.3 (H_2, 20% $Pd(OH)_2$/C, HCl in ^{i}PrOH) provided UDP-Gal*f* analogues (1*R*)-**9a–c** as their HCl

salts (86-100%). Not unexpectedly, the iodine moiety in compound (1*R*)-**8c** was removed during the hydrogenolysis step. In case of pyrido[3,2-*d*]pyrimidyl-arabinitol (1*R*)-(*S*$_R$)-**7d**, removal of the chiral sulfinyl group was accomplished using *in situ* generated HCl in MeOH. The benzyl protecting groups were subsequently cleaved by treatment with excess of boron trichloride (2 × 10 equiv.) without isolating the intermediate. Compound (1*R*)-**9d** (as its HCl salt) was obtained in 36% (2 steps) after purification through C18 reversed-phase flash chromatography (see Eq. 1).



Equation 1. Preparation of (1*R*)-**9d**.

To further diversify and extend the scope of our methodology, we also generated more elaborated imino-based UDP-Gal*f* analogues using additional simple protocols. (1*R*)-(*S*$_R$)-**9e** was thus prepared (53%, un-optimized reaction) by reacting 4-iodophenyl-triazolyl-imino-L-arabinitol (1*R*)-(*S*$_R$)-**7c** with 6-methoxy-3-pyridinylboronic acid (1.0 equiv.), K_2CO_3 (2.0 equiv.) and tetrakis(triphenyl-phosphine)palladium(0) (10 mol%) in toluene at 80 °C (see (1*R*)-(*S*$_R$)-**7e**, ESI). Following GP1 and GP.3 (1*R*)-**9e** as its HCl salt, was obtained in moderate yield (44% over 2 steps, Scheme 2).



Scheme 2. Preparation of (1*R*)-**9e**.

Inhibition of GlfT2

With these compounds in hand, each was tested as a potential inhibitor of GlfT2 using a reported coupled spectrophotometric assay, which employs a trisaccharide acceptor substrate and UDP-Gal*f* as the donor.[20]

To determine an initial indication of inhibitory potential, each compound was evaluated at 4 mM concentration with the acceptor at 2 mM and donor at 3 mM. Under these conditions, four of the compounds – **9b**, **9c**, **9d** and **9e** – nearly completed inhibited the enzyme (Table 1).

Table 1. Evaluation of **9a**–**9e** as inhibitors of mycobacterial GlfT2.

Compound	% Inhibition at [4 mM][a]	IC_{50} (mM)
9a	30	ND[b]
9b	100	0.8 ± 0.1
9c	99	0.9 ± 0.0
9d	99	1.6[c]
9e	100	2.4 ± 0.0

[a] Acceptor substrate was present at 2 mM and donor (UDP-Gal*f*) at 3 mM. [b] Not Determined. [c] Shape of inhibition curve precluded determination of a standard deviation.

Interestingly, **9a**, which carries a methoxyphenyl substituent, is only weakly active (~30% inhibition) against the enzyme. It thus appears that electron rich substituents on the aromatic ring in compounds of this type are not well tolerated by the enzyme.

A more accurate estimation of the potency of **9b**, **9c**, **9d** and **9e** as inhibitors of GltT2 was obtained by measuring IC_{50} values (Table 1). The four compounds inhibited the enzyme over an IC_{50} range of 0.8–2.4 mM, with the most potent being those with the phenyl or *p*-trifluromethylphenyl substituents.

Therefore, electron-neutral or electron-deficient substituents on the aryl ring lead to the most potent inhibition. It should be noted that although in absolute terms these activities are relatively weak, **9b** and **9c** are among the most potent GlfT2 inhibitors reported to date. Earlier work using modified sugar nucleotide analogs have identified GlfT2 inhibitors with low micromolar potency.[21] However, such molecules are of limited utility as therapeutic leads. Attempts to identify inhibitors of GlfT2 that are not based on sugar nucleotides including other iminosugars, have produced more modest results with IC_{50} values typically exceeding 3 mM.[22]

In light of these results, we postulate that additional modification of the L-*arabino* 1-*C*-iminopentitol scaffold may provide compounds with increased potency as GlfT2 inhibitors.

Conclusions

In summary, we report herein preliminary results on a method to generate rapidly 1-C-substituted imino-L-arabinitol

scaffolds tethered to 1,2,3-triazolyl appendages as simplified UDP-Gal*f* mimics. This approach, which is more convenient than our earlier zinc mediated propargylation protocol,[13] involves stereoselective addition of 3-(trimethylsilyl)-2-propynyl magnesium bromide to (S_R)-*N*-*tert*-butanesulfinyl-α/β-D-xylofuranosylamine (S_R)-**2**, followed by mesylation, cyclization, and deprotection sequences. To illustrate the methodology, five compounds were prepared (e.g., (1*R*)-**9a**–**e**) as potential inhibitors of GlfT2. Compounds **9b** and **9c** showed sub-millimolar inhibition of GlfT2 and represent two of the most active non-sugar nucleotide based inhibitors of the enzyme reported to date. The synthesis of additional L-*arabino* 1-*C*-iminopentitols bearing triazoles functionalized with electron-deficient aryl substituents (e.g., nitrophenyl, dinitrophenyl) may provide even more potent inhibitors of the enzyme. The availability of a compound such as **6** opens access to a great diversity of iminosugar–heterocycle conjugates by click chemistry, which could enter high-throughput screening towards a diversity of biological targets.

Experimental Section

General Remarks. Unless otherwise stated, all reagents were purchased from commercial sources and used as received. 2,3,5-tri-*O*-benzyl-D-xylofuranose,[23] 2,3,5-tri-*O*-benzyl-*(S_R)-N-tert*-butanesulfinyl-α/β-D-xylofuranosylamine (S_R)-**2**,[15] tetrakis(triphenylphosphine)palladium(0),[24] and (3-(trimethylsilyl)prop-2-yn-1-yl)magnesium bromide,[25] were prepared following literature procedures. $Ti(OEt)_4$ (50 mL in glass bottle), 4 Å activated molecular sieves (pellets, 1.6 mm diameter), toluene (puriss. p.a., ACS reagent, ≥ 99.7% (GC)), diethyl ether (puriss. p.a., ACS reagent, ≥ 99.8 %) with 2,6-di-*tert*-butyl-4-methylphenol (~ 10 ppm) as inhibitor, THF (99.9% GC) with 2,6-di-*tert*-butyl-4-methylphenol (250 mg/L) as stabilizer, methanol (anhydrous, 99.8%) and Amberlite® IRA-400 (Cl^- form) were purchased from Sigma-Aldrich. Ethyl acetate (EA) (99.8% GC, tech. grade, pure for synthesis), petroleum ether (PE) (tech. grade, pure for synthesis, bp 40-65 °C) and dichloromethane (99.95% GC, tech. grade, pure for synthesis, stab. with ethanol) were purchased from Carlo Erba. Magnesium turnings were activated in an oven at 150 °C (24 h) and allowed to reach room temperature over $CaCl_2$ in a dessicator, prior to use. (3-(trimethylsilyl)prop-2-yn-1-yl)magnesium bromide was titrated using salicylaldehyde phenylhydrazone as indicator.[26] Toluene (puriss. p.a., ACS reagent, ≥ 99.7% (GC)) and THF (99.9% GC) with 2,6-di-*tert*-butyl-4-methylphenol (250 mg/L) as stabilizer were purified by passage through a column containing activated alumina under nitrogen pressure (Dry Solvent Station GT S100, GlassTechnology, Geneva, CH). Dichloromethane (99.99% GC) and diethyl ether (puriss. p.a., ACS reagent, ≥ 99.8 %) with 2,6-di-*tert*-butyl-4-methylphenol (~ 10 ppm) as inhibitor were distilled from calcium hydride. 4 Å MS was activated by drying in an oven at 500°C (48 h). It was then allowed to reach room temperature and kept over $CaCl_2$ in a desiccator prior to use. Amberlite® IRA-400 was prepared in its OH^- form by passing 1M KOH until the effluent is free from chloride ions, then washed with distilled H_2O until neutral pH and washed further with MeOH. NMR spectra were recorded at 298 K with a Bruker Avance III HD nanobay 400 MHz spectrometer equipped with a BBO probe. The nuckei-signal assignments were done with the aid of 1 D [^{1}H NMR, ^{13}C NMR, Distortionless Enhancement by Polarization Transfer (DEPT)] and 2 D Correlation Spectroscopy [(^{1}H–^{1}H COSY and ^{1}H–^{13}C Heteronuclear Single Quantum Coherence (HSQC)] experiments. When appropriate or in case of ambiguous proton and carbon, assignments were established using Nuclear Overhauser Effect Spectroscopy (NOESY). ^{1}H NMR (400 MHz) chemical shift values are listed in parts per million (ppm) downfield from TMS as the internal standard or relative to the corresponding non-deuterated solvent. Data are reported as follows: chemical shift (ppm on the δ scale), multiplicity (s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublet, t = triplet, q = quadruplet and m = multiplet), coupling constant *J* (Hz), and integration. ^{13}C NMR (101 MHz) chemical shifts are given in ppm relative to the corresponding non-deuterated solvent or TMS as the internal standard. High-resolution mass spectra were recorded with a MaXis ESI qTOF ultrahigh-resolution mass spectrometer (FR2708, Orléans). Infrared spectra were recorded with a Thermo Scientific Nicolet IS10 FTIR spectrometer using diamond ATR golden gate sampling and are reported in wave numbers (cm^{-1}). Specific optical rotations were measured with a Perkin–Elmer 341 polarimeter in a thermostated (20 °C) 1 dm long cell with high-pressure sodium lamp and are reported as follow: $[\alpha]_D^T$ [solvent, c (g/100 mL)]. Analytical thin-layer chromatography (TLC) was performed with Merck Silica Gel 60 F254 precoated plates. Visualization of the developed chromatogram was performed under ultraviolet light (254 nm) and on staining by immersion in aqueous, acidic ceric ammonium molybdate (CAM; 470 mL H_2O, 28 mL H_2SO_4, 24 g ammonium molybdate, 0.5 g cerium ammonium nitrate) followed by heating on a hot plate. Normal phase flash chromatography was performed in air on Silica Gel 60 (230–400 mesh) with petroleum ether (PE, bp 40–65 °C) and ethyl acetate (EA) as eluents, unless otherwise stated. HPLC analysis of compound (1*R*)-**9e** was performed on a C18 Zorbax Eclipse plus column (Agilent), 2.1 mm diameter, 150mm length and 5 µm particle size with a 0.4 $mL.min^{-1}$ flow rate. The compound was eluted with a 15 minutes gradient from 70:30 water/methanol with 0.1 % (v/v) formic acid to 100% methanol with 0.1% formic acid.

General Procedure for the Cleavage of the N-Sulfinyl Protecting Group (GP. 1). In a single-necked round-bottomed flask under argon atmosphere was inserted AcCl and dry methanol and the solution was stirred at room temperature (ca. 20 °C) for 30 minutes (solution A). Another single-necked flask under argon atmosphere was charged with related *N*-sulfinyl-protected imino-L-arabinitols and solution A was added dropwise through syringe. The reaction mixture was stirred for 30 min and neutralized by addition of Amberlite® IRA-400 ion-exchange resin (OH^- form) until pH 7-8. The solution was filtered through a cotton plug and concentrated under vacuum to afford, after purification through SiO_2-column chromatography, corresponding N-deprotected compound.

General Procedure for the CuAAC (GP. 2). A single-necked round-bottomed flask under air atmosphere was charged with arabinitol (1*R*)-(S_R)-**6**, the related azido compound **a-d** and DMF (flask A). Then, copper (II) sulphate pentahydrate and sodium L-ascorbate were dissolved in dist. H_2O and added to A and the reaction mixture was stirred at 20 °C, until no more starting material was present (TLC analysis). The mixture was quenched by addition of sat. *aq.* NaCl and extracted three times with CH_2Cl_2. Combined organic layers were dried ($MgSO_4$) and concentrated under vacuum. The crude residue was purified by column chromatography to afford triazolyl-imino-L-arabinitol (1*R*)-(S_R)-**7a-d**.

General Procedure for Hydrogenolysis of Compounds (1*R*)-8a-c and (1*R*)-8e (GP. 3). A single-necked round-bottomed flask was charged with (1*R*)-**8a-c** or (1*R*)-**8e**, aq. HCl (4 equiv.) and *i*-PrOH. The solution was degassed under vacuum and filled with argon (Ar-filled balloon) five times. 20% palladium hydroxide on carbon was added and the suspension was degassed and saturated with H_2 (H_2-filled balloon) five times. The reaction mixture was then stirred at 20 °C under H_2 atmosphere (balloon of H_2) for a given time and filtered over millipore membrane (0.2 µm). The cake was rinsed with MeOH and solvents were evaporated under reduced pressure. Removal of *i*-PrOH was carried out by co-evaporating

(ten times) the crude mixture with MeOH to give (1*R*)-**9a-c** and (1*R*)-**9e** respectively.

Biological Assays. Methods.

Spectrophotometric assays were performed in 384-array microtiter plate wells containing 18.5 mM MOPS, pH 7.6, 50 mM KCl, 20 mM $MgCl_2$, 2.2 mM NADH, 7.0 mM PEP, 15 U pyruvate kinase (PK, EC 2.7.1.40) and 33.6 U lactate dehydrogenase (LDH, EC 1.1.1.27). MOPS, pH 7.6, was added as a 20-fold stock solution.[20] KCl, $MgCl_2$, the acceptor and donor substrates were dissolved in de-ionized distilled water (MQ). All other assay components were prepared in 50 mM MOPS, pH 7.6, with the exception of PEP, which, due to its acidity, was buffered in 250 mM MOPS, pH 7.6. Stock solutions of NADH, PEP, PK, LDH and donor were made fresh on day of use and stored on ice. A standard assay reaction contained UDP-Gal*f*[27] at a final concentration of 3 mM and acceptor trisaccharide (octyl β-D-galactofuranosyl-(1 →5)-β-D-galactofuranosyl-(1 →6)-β-D-galactofuranoside) at 2 mM.[28] Each reaction was initiated by addition of 0.036 µg GlfT2 (7.5 µg/mL) to the assay mixture, bringing the final assay volume to 20 µL. Reactions were incubated at 37 °C and monitored at 340 nm at 10^{-15} sec intervals using a Spectra Max 340PC microplate reader. The velocities were determined from the decrease in NADH absorbance at 340 nm and expressed as a percentage of a control rate in the absence of inhibitor. Data were graphed and analyzed using GraphPad Prism® Version 4. On the first day of use, UDP-Gal*f* analogs were dissolved in MQ to give 40 mM stock solutions. Analogs were initially screened in duplicate at a final concentration of 4 mM. Compounds that showed greater than 95% inhibition were tested at various concentrations from 0.2 to 6.0 mM to determine the IC_{50} value. Duplicates for each concentration were carried out.

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Keywords: Synthetic methods • Iminosugar-*C*-Glycosides • [3+2] Cycloadditions • UDP-Gal*f* mimics • *Mycobacterium tuberculosis*

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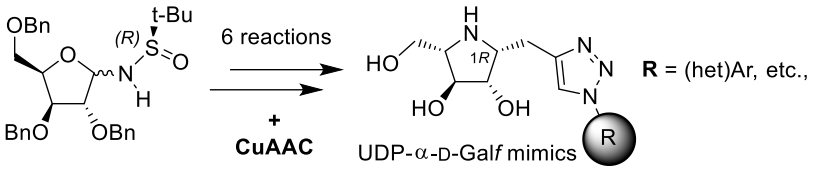

The convenient preparation of 1-*C*-propargyl-imino-L-arabinitol derivatives coupled via a triazole-tether to various aromatic and heteroaromatic groups is reported. These structures that can be considered as simplified mimics of UDP-Gal*f*, the substrate of GlfT2, a galactofuranosyltransferase present in mycobacteria were evaluated as inhibitors of the enzyme and found to have significant activity.

Iminosugars, Synthesis

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Triazole-Linked Iminosugars and Aromatic Systems as Simplified UDP-Gal*f* Mimics: Synthesis and Preliminary Evaluation as Gal*f*-transferase Inhibitors