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Lewis-X-Containing Triterpenoid Saponins Inhibit DC-SIGN- and L-SIGN-Mediated Transfer of HIV-1 Infection

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Blocking dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN)- and liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN)-mediated human immunodeficiency virus 1 (HIV-1) attachment to immune cells represents a promising strategy for developing antiretroviral agents effective during the early stages of sexual transmission. Although mannose- and fucose-based ligands have received considerable attention, Lewis-based inhibitors remain relatively underexplored. In this study, we report the first synthesis of Lewis-X-containing triterpenoid saponins featuring betulinic acid and echinocystic acid as aglycones. These saponins were stereoselectively and efficiently synthesized in six linear steps using a convergent approach that leveraged thioglycoside and trichloroacetimidate glycosylation chemistries. Notably, our findings demonstrate that these Lewis-X-containing triterpenoid saponins are among the most potent monovalent inhibitors reported to date of DC-SIGN- and L-SIGN-mediated transfer of HIV-1 infection to CD4-positive cells, with IC_{50} values in the low micromolar range (21–50 μM). This work lays a valuable foundation for the development of saponin-based antiviral agents targeting immune cells.

1. Introduction

Human immunodeficiency virus 1 (HIV-1) infection remains a significant global health burden, accounting for substantial morbidity and mortality worldwide. Although current antiretroviral therapy has significantly improved survival rates among people living with HIV, it falls short of completely eradicating the virus from the body.[1] Moreover, antiretroviral therapy can lead to undesirable side effects, including residual inflammation and long-term toxicity. Therefore, novel antiviral strategies targeting HIV-1 are urgently needed, particularly those focusing on blocking the initial stages of viral infection, which would represent a critical step forward in combating this devastating disease.[2]

Dendritic cells (DCs) are pivotal antigen-presenting cells bridging the innate and adaptative immune systems. Immature DCs efficiently capture microbial pathogens, process them, and present antigens to T cells via the major histocompatibility complex, initiating both cellular and humoral immune responses.[3] Depending on their tissue localization and differentiation state, DCs respond to diverse microbial threats by expressing specific C-type lectins on their surface.[4] These lectins include DC-SIGN (dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin), Langerin, DEC-205, and the mannose receptor.[5]

DC-SIGN and its structural homolog L-SIGN (liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin) are transmembrane, calcium-dependent carbohydrate-binding proteins that serve as pathogen-recognition receptors.[6] The extracellular domains of these C-type lectins contain a carbohydrate-recognition domain that recognizes mannose-containing glycoconjugates and fucose-containing Lewis-blood-group antigens, including Lewis-X (α-L-Fuc*p*-(1→3)-[β-D-Gal*p*-(1→4)]-D-GlcNAc*p*, specific to DC-SIGN),[7] Lewis-Y, Lewis-A, and Lewis-B (Figure 1).[8] Various highly glycosylated viruses bind to DC-SIGN through interactions with their envelope glycoproteins.[9] For instance, the HIV-1 envelope glycoprotein gp120, bearing high-mannan epitopes [$(Man)_9(GlcNAc)_2$], interacts with DC-SIGN,[10] protecting the virus from intracellular degradation. This binding enables virus particles to remain infectious for several days and efficiently transmit to CD4-positive T lymphocytes upon contact, a process known as infection in *trans* (Figure 2B).[11] This facilitates access to lymphoid tissue, where primary replication occurs, and promotes evasion of immune defenses. Consequently, blocking DC-SIGN-mediated HIV-1 attachment offers a promising strategy for developing antiretroviral

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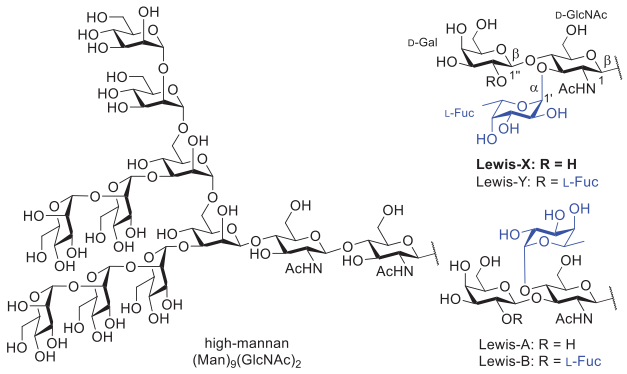


Figure 1. Structures of high-mannan and Lewis-type sugars that bind to DC-SIGN.

agents effective in the early stages of sexual transmission (Figure 2C).[12]

Over the past two decades, significant efforts have focused on designing selective and potent DC-SIGN inhibitors,[13] encompassing monovalent glycomimetics,[14] multivalent presentation of saccharides/glycomimetics,[15] and noncarbohydrate[16] antagonists. Fucose-based ligands have garnered attention due to the higher specificity of DC-SIGN for L-fucose (K_i = 6.7 mM) compared to D-mannose (K_i = 13.1 mM),[8a] as well as the potential for D-mannose to interact with Langerin, which may promote beneficial viral internalization and degradation.[17] Despite progress in developing fucose-based glycomimetic antagonists,[14a,b] Lewis-based inhibitors remain understudied. Notably, Lewis-X epitopes, commonly found in human milk, inhibit HIV-1 transfer to CD4-positive T lymphocytes when presented in oligo/polymeric forms or conjugated to proteins, but not as free Lewis-X.[18] A seminal study by van Kooyk and coworkers[15c] demonstrated that Lewis-X-bearing poly(amidoamine) dendrimers selectively block DC-SIGN/gp120 interactions, effectively inhibiting HIV-1 transmission to CD4-positive T cells.

Betulinic acid (BetA) is a relatively nontoxic,[19] bioactive lupane-type triterpenoid found in various vegetal species.[20] BetA has demonstrated inhibition of HIV-1 maturation,[21] with its derivative, bevirimat (PA-457), advancing to phase 2 clinical trials.[22] BetA derivatives have shown broad antiviral activity against Dengue,[23] influenza,[24] and SARS-CoV-2[25] viruses. Notably, lupane-type triterpenoid saponins[26] exhibit reduced hemolytic activity compared to saponins derived from other triterpenoids with similar sugar residues.[27] Echinocystic acid (EchA), an oleanane-type triterpenoid, has also attracted attention as an antiviral agent[28] and EchA-based saponins have been found to exhibit immunostimulatory properties.[29]

Leveraging the inherent antiviral activity and low toxicity of BetA and EchA, we hypothesized that conjugating a Lewis-X trisaccharide epitope to the triterpenoid scaffold would generate DC-SIGN antagonists, inhibiting viral transfer infection (Figure 2C). Here, we report the first synthesis of chimeric Lewis-X-containing triterpenoid saponins **1** and **2** through a convergent and high-yielding glycosylation approach (Figure 2A). We demonstrate that triterpenoid saponins **1** and **2** are among the most potent monovalent inhibitors of both DC-SIGN- and

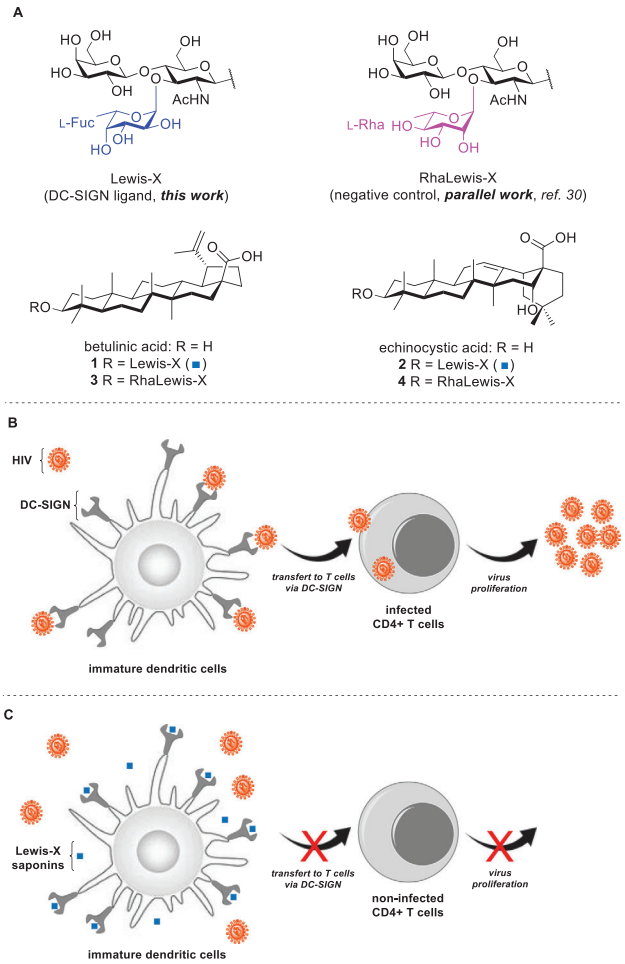


Figure 2. (**A**) Structures of the Lewis-X- and RhaLewis-X-containing saponins (**1**—**4**). (**B**) Schematic representation of DC-SIGN-mediated HIV-1 *trans*-infection to CD4-positive cells. (**C**) Schematic representation of the inhibition of DC-SIGN-mediated HIV-1 *trans*-infection to CD4-positive cells by Lewis-X-containing saponins **1** and **2**.

L-SIGN-mediated HIV-1 transfer to CD4-positive cells reported to date. In our first parallel study,[30] we detail the synthesis of analogues **3** and **4** (Figure 2A), replacing α-L-fucopyranose with α-L-rhamnopyranose, through both iterative and convergent glycosylation pathways. These nonnatural saponins were intended to be used as negative controls for the antiviral assays. Furthermore, as described in our second parallel work,[31] triterpenoid saponins **1**–**3** were evaluated for their in vivo toxicological and immunological potential in both wild-type and hDC-SIGN transgenic mice.

2. Results and Discussion

2.1. Synthetic Strategy

We designed triterpenoid saponins **1** and **2** to feature the Lewis-X trisaccharide at the C3 position, leaving the carboxylic acid free at the C28 position to enhance aqueous solubility and/or append other epitopes.[32] Our retrosynthetic strategy (Figure 3)

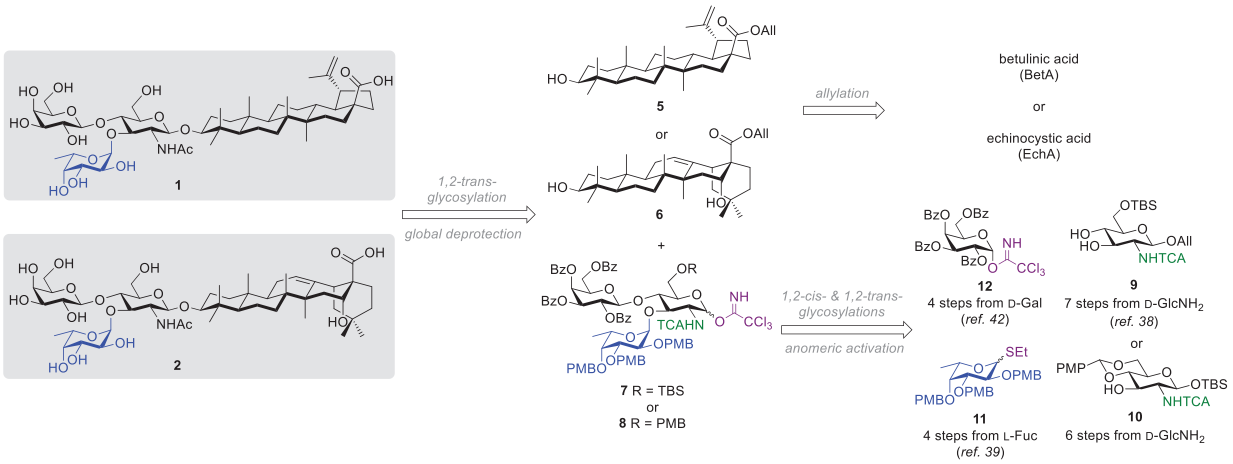


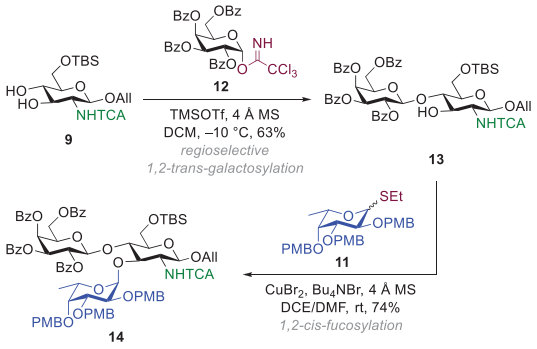
Figure 3. Retrosynthetic analysis of Lewis-X-containing saponins **1** and **2** via a convergent glycosylation strategy.

involves covalently linking the C3 hydroxyl group of BetA or EchA to the reducing end of the GlcNAc residue through a β-glycosidic bond, mimicking natural Lewis-X oligomers. We adopted a convergent approach over an iterative one, capitalizing on a "universal" and suitably protected Lewis-X glycosyl donor, to couple with allyl betulinate (**5**)[33] or allyl echinocystate (**6**)[34] (Scheme S4), which would yield target saponins **1** and **2** upon global deprotection. The 1,2-*trans*-stereoselectivity of the glycosylation would be directed by the neighboring participation of an *N*-trichloroacetamide (NHTCA) group.[35] Two Lewis-X glycosyl donors (**7** and **8**) were designed, varying at the C6 position of the GlcNAc residue (TBS or PMB). Their anomeric group would be activated as Schmidt's trichloroacetimidates (TCA),[36] renowned for high yields and versatility in the synthesis of triterpenoid saponins.[26a,37] The synthesis of the trisaccharide would commence from either GlcNAc diol **9**[38] (Scheme S1) or *para*-methoxybenzylidene derivative **10**, prepared in seven and six steps from D-glucosamine hydrochloride, respectively. Trisaccharide **7** would result from regioselective 1,2-*trans*-galactosylation at the C4 position of GlcNAc derivative **9**, followed by 1,2-*cis*-fucosylation using thioethyl donor **11**[39] (Scheme S3) under Lemieux anomerization conditions, involving S_N2 displacement of an in situ-formed transient β-bromide intermediate.[40] Conversely, trisaccharide **8** would be obtained through initial fucosylation at the C3 position of derivative **10**, regioselective opening of the *para*-methoxybenzylidene, and subsequent stereoselective galactosylation at the C4 position.

Protecting groups were chosen to minimize side reactions on the triterpenoid scaffold during later deprotection. Specifically, the use of benzyl (Bn) groups requiring hydrogenolysis was avoided to prevent double bond reduction. Strongly acidic reaction conditions were also avoided to prevent the so-called Wagner-Meerwein rearrangement of the lupane scaffold to germanicane, particularly during the glycosylation reactions.[41]

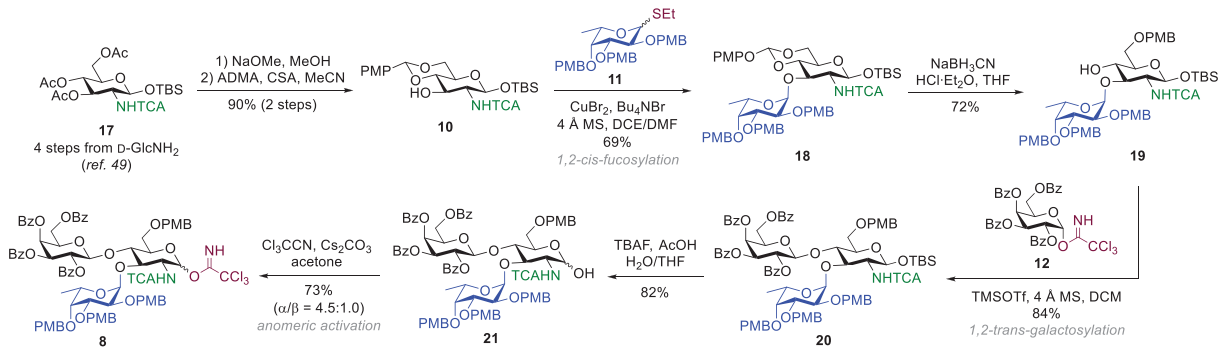
2.2. Synthesis of Lewis-X Glycosyl Donor

We initiated our synthetic strategy by preparing allylated Lewis-X trisaccharide **14** (Scheme 1). Diol GlcNAc acceptor **9** was coupled with perbenzoylated TCA galactosyl donor **12**[42] (Scheme S2) under the catalysis of TMSOTf, providing disaccharide **13** in 63% yield. The regioselectivity was confirmed by 2D HMBC NMR, showing ^3J interactions between Gal-C1" and GlcNAc-H4, and Gal-H1" and GlcNAc-C4. This regioselectivity is attributed to the TBS group at C6, hindering the upper side of the pyranose, and the electron-withdrawing NHTCA group at C2, reducing the nucleophilicity of the hydroxyl group at C3. These combinative effects favorably direct the glycosylation toward the hydroxyl group at C4.[43] Next, we introduced the fucose residue at C3 using Lemieux anomerization conditions, coupling disaccharide acceptor **13** with thioethyl fucosyl donor **11**, protected by nonparticipating PMB groups, under the promotion of $CuBr_2$/Bu_4NBr,[44] furnishing fully protected Lewis-X trisaccharide **14** in 74% yield. The α-anomeric configuration of the fucose residue was confirmed by ^{1}H NMR, based on the coupling constant of the anomeric proton ($^3J_{H1',H2'} = 3.5$ Hz).



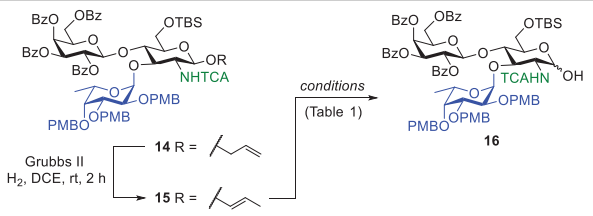
Scheme 1. Synthesis of trisaccharide **14** bearing an allyl group at the anomeric position.

With trisaccharide **14** in hand, we tried to remove the allyl group at the reducing anomeric position. However, the interference of the NHTCA group represented a challenge in finding suitable reaction conditions. Conventional deallylation approaches under Ir-catalyzed reactions[45] failed to yield desired hemiacetal **16**. In our first parallel publication,[30] we demonstrated that anomeric deallylation of an NHTCA-protected GlcNAc monosaccharide was possible using Grubbs'



Scheme 2. Synthesis of Lewis-X trisaccharide TCA donor **8**.

Table 1. Attempts to cleave the allyl group at the anomeric position of trisaccharide **14**.



Entry	Conditions	Yield[a] [%]
1	I_2, H_2O, THF/py, rt, 48 hours	11[b,c]
2	I_2, THF/H_2O, rt, 16 hours; *then* 45 °C, 1 hour	28[b,c]
3	NBS, THF, rt; *then* H_2O, rt	nd[c]
4	NIS, THF, rt; *then* H_2O, rt to 45 °C	nd[c]
5	*m*CPBA, DCM, 0 °C; *then* Et_3N, 0 °C	nd[c]
6	OsO_4, $NaIO_4$, dioxane/H_2O 3:1, rt	nd[c]

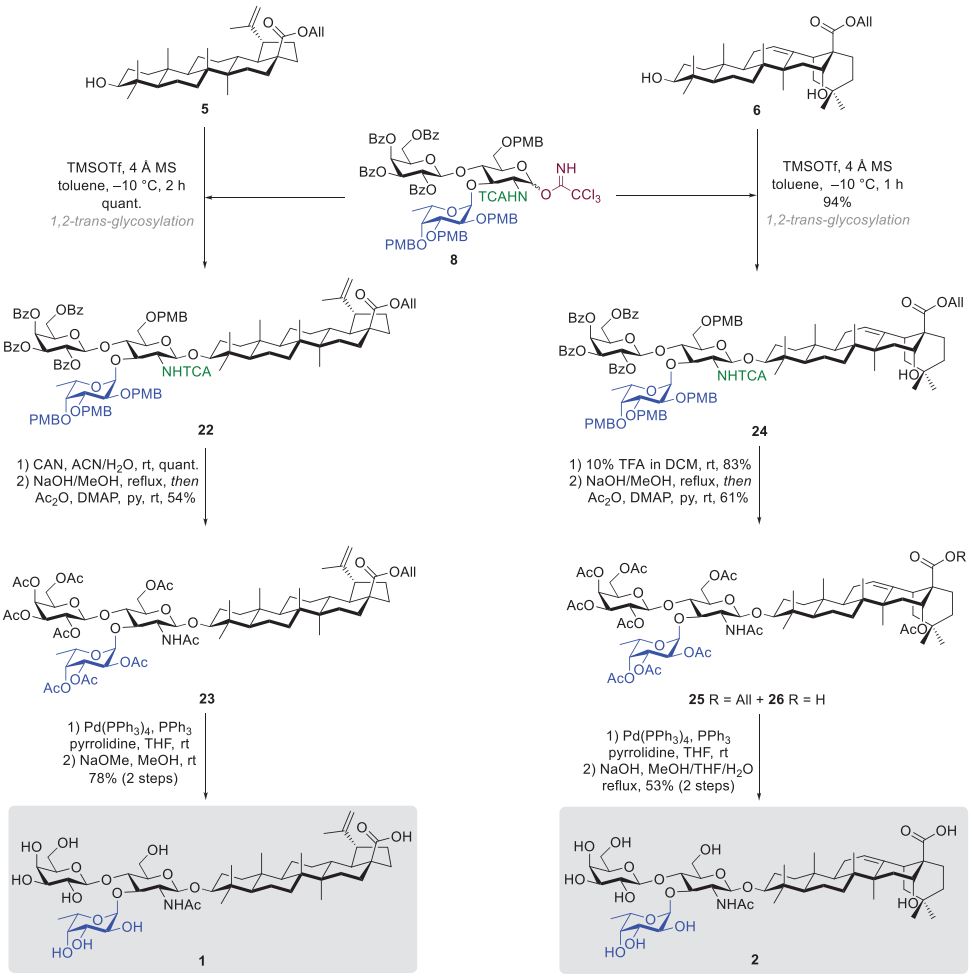
[a] Isolated yields over two steps from trisaccharide **14**.
[b] Partial degradation.
[c] Not detected (decomposition of starting material).

second-generation catalyst in the presence of hydrogen,[46] inducing isomerization and formation of 1-propenyl derivative **15**. Subsequent hydrolysis using iodine in water allowed the formation of the corresponding hemiacetal in convenient yields. Unfortunately, applying these conditions to trisaccharide **14** gave hemiacetal **16** in low yield over two steps (11%, Table 1, entry 1). TLC analysis revealed partial degradation of the starting material. Increasing the temperature slightly improved the yield to 28% (entry 2), with concomitant partial degradation of trisaccharide **15**. Alternative attempts using NBS, NIS, or oxidative reagents (*m*CPBA and OsO_4/$NaIO_4$) resulted in complete degradation of the starting material (entries 3 to 6).

In light of these unsatisfying results, and inspired by Schmidt's works on Lewis-A antigens,[47] we addressed the deallylation issue by modifying the protecting groups on the GlcNAc residue (Scheme 2). GlcNAc derivative **17**, bearing a TBS group at the anomeric position, was prepared from glucosamine hydrochloride in four steps, as previously described (Scheme S1).[48] Zemplén deacetylation followed by installation of a *para*-methoxybenzylidene group to protect the C4 and C6 positions provided GlcNAc acceptor **10** in 90% yield over two steps. α-Fucosylation at position C3 proceeded smoothly under $CuBr_2$/Bu_4NBr conditions, generating disaccharide **18** in 69% yield. Notably, purification required slightly basic conditions (Et_3N in the eluent) to prevent degradation of compound **18** caused by the in situ formation of HBr over the silica gel. Regioselective ring opening of the *para*-methoxybenzylidene group using $NaBH_3CN$ and HCl released the OH at position C4,[49] providing disaccharide acceptor **19** in 72% yield. Galactosylation at position C4 under Schmidt's conditions furnished fully protected Lewis-X trisaccharide **20** in 84% yield. Anomeric cleavage of the TBS group was achieved in 82% yield using tetrabutylammonium fluoride (TBAF) buffered with acetic acid to prevent undesired benzoyl group cleavage. Finally, hemiacetal **21** was activated by inserting a TCA group at the anomeric position, providing fully protected Lewis-X glycosyl donor **8** as an α/β mixture (4.5:1.0) in 73% yield.

2.3. Synthesis of Lewis-X-Containing Triterpenoid Saponins

With a reliable approach to access substantial amounts of Lewis-X donor **8**, we proceeded to glycosylate the C3 position of BetA and EchA (Scheme 3). Allyl betulinate **5**[33] was coupled with glycosyl donor **8** using TMSOTf in toluene at −10 °C, cleanly furnishing fully protected saponin **22** in a quantitative yield. Careful catalyst addition was crucial as <0.15 equiv of TMSOTf led to undesired oxazoline formation,[50] reducing the glycosylation yield. The stereoselectivity of the newly formed glycosidic bond was confirmed by 2D undecoupled HSQC NMR, showing a $^1J_{C1,H1}$ coupling constant of 160 Hz, indicative of the axial orientation of the anomeric proton.[51] The four-step deprotection sequence began with PMB cleavage using CAN in H_2O/MeCN, furnishing the corresponding tetraol (**S4**, see SI) in quantitative yield. Saponification under strongly basic conditions (NaOH/MeOH, reflux) removed benzoyl and NHTCA groups, sparing the hindered allyl ester at the C28 position of BetA. Acetylation enabled the installation of the native NHAc group, giving fully acetylated saponin **23** in 54% yield over three steps. Final deallylation at the carboxylic position using $Pd(PPh_3)_4$ and pyrrolidine,[52] followed by Zemplén deacetylation, provided target BetA saponin **1** in 78% over two steps.



Scheme 3. Synthesis of Lewis-X-containing triterpenoid saponins **1** and **2**.

EchA saponin **2** was obtained following a similar approach (Scheme 3). Glycosylation of allyl echinocystate **6**[34] with Lewis-X glycosyl donor **8** gave fully protected saponin **24** in 94% yield. PMB deprotection using 10% TFA in DCM provided the desired tetraol intermediate (**S5**, see SI) in 83% yield. Using classical PMB deprotection methods (CAN or DDQ) resulted in the degradation of the starting material. As confirmed by HRMS, saponification followed by acetylation provided a mixture of allylated saponin **25** and deallylated saponin **26**. Partial deallylation was attributed to the higher lability of the allyl ester in EchA than BetA. Final deallylation and saponification provided target saponin **2** in 53% yield over two steps. Strongly basic conditions were used to cleave the acetyl group found at the hindered C16 position of EchA.

Lewis-X-containing BetA and EchA saponins (**1** and **2**) were obtained in overall yields of 42 and 25%, respectively, over six linear steps from Lewis-X donor **8** and the corresponding allyl betulinate (**5**) or allyl echinocystate (**6**). The homogeneity of the synthetic saponins was confirmed by 1D and 2D NMR and HRMS analyses (see SI for data). Similar analytical characterizations were performed for RhaLewis-X-containing saponins **3** and **4**, as reported in our first parallel publication.[30]

2.4. Inhibition of DC-SIGN- and L-SIGN-Mediated Transfer of HIV-1 Infection by Saponins

Lewis-X-containing saponins **1** and **2** were evaluated in vitro for their inhibition of DC-SIGN-mediated HIV-1 *trans*-infection. For comparison, saponins **3** and **4** bearing a trisaccharide with rhamnose instead of fucose, as well as triterpenoid aglycones (BetA and EchA) and free Lewis-X (LeX), were also assessed (Figure 4, top). Since L-SIGN can enhance HIV-1 infection, the compounds were also evaluated against this lectin (Figure 4, bottom). To investigate this, DC-SIGN+ and L-SIGN+ 3T3 cells were serially titrated with the compounds (starting from 200 µM) and pre-incubated for 30 minutes before adding different replication-competent HIV strains (BF520, JRCSF, Z331M, CH077.t, and CH505). Notably, all virus strains were derived from transmitted/founder (T/F) strains, except JRCSF, which derives from chronic infection. Following a four-hour incubation and washing, TZM-bl cells, engineered to express HIV-1 receptor CD4 and co-receptor CCR5 with an integrated firefly luciferase reporter gene, were co-cultured with the 3T3 cells. After three days, cells were lysed and a luciferase assay was performed to measure HIV-1 infection.

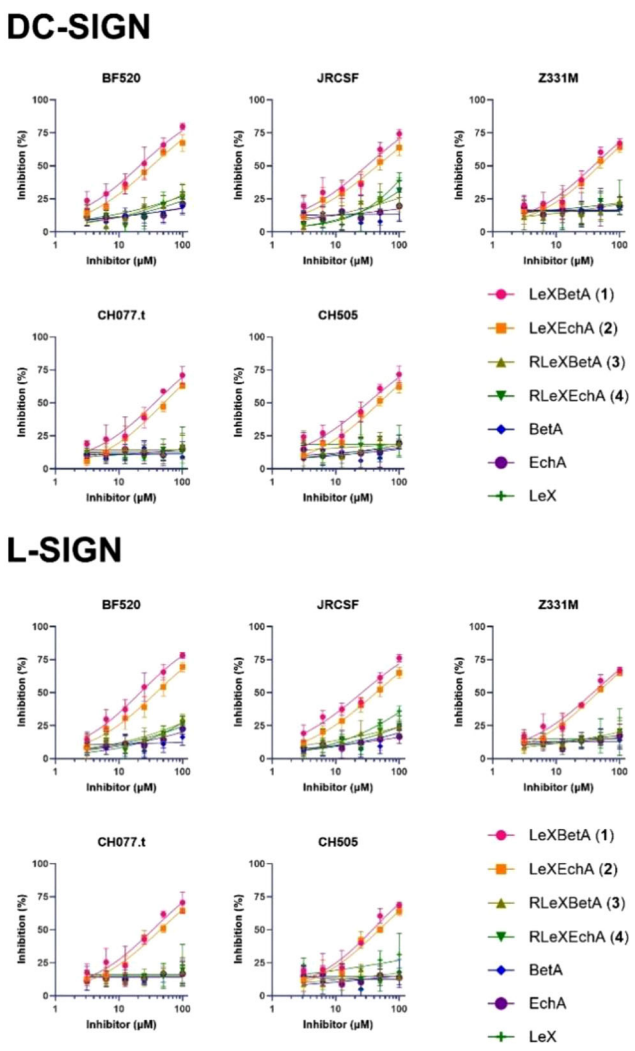


Figure 4. Evaluation of DC-SIGN- (top) and L-SIGN- (bottom) mediated inhibition of HIV-1 transfer to CD4-positive cells by synthetic triterpenoid saponins **1**–**4**, triterpenoids (BetA and EchA), and free Lewis-X (LeX). RLeX: RhaLewis-X.

Table 2. DC-SIGN- and L-SIGN-mediated inhibition of HIV-1 transfer.

	IC_{50} [μM][a] DC-SIGN (top)/L-SIGN (bottom)				
Cpd	BF520	JRCSF	Z331M	CH077.t	CH505
1	22 ± 3 21 ± 2	33 ± 5 26 ± 2	41 ± 6 40 ± 6	39 ± 6 36 ± 6	34 ± 6 45 ± 4
2	32 ± 3 38 ± 4	45 ± 7 43 ± 4	50 ± 6 45 ± 4	49 ± 5 45 ± 4	48 ± 6 49 ± 6
3	>100 >100	>100 >100	>100 >100	>100 >100	>100 >100
4	>100 >100	>100 >100	>100 >100	>100 >100	>100 >100
BetA	>100 >100	>100 >100	>100 >100	>100 >100	>100 >100
EchA	>100 >100	>100 >100	>100 >100	>100 >100	>100 >100
Lewis-X	>100 >100	>100 >100	>100 >100	>100 >100	>100 >100

[a] IC_{50} values ± SEM.

Remarkably, synthetic triterpenoid saponins **1** and **2**, bearing a Lewis-X trisaccharide at their C3 position, inhibited DC-SIGN-mediated HIV-1 *trans*-infection to CD4-positive cells in a dose-dependent manner. As hypothesized, control saponins **3** and **4**, triterpenoids BetA and EchA, and free Lewis-X trisaccharide showed low or no inhibitory activity, underscoring the critical role of both the triterpenoid scaffold and the Lewis-X epitope in mediating interactions with DC-SIGN. Notably, BetA saponin **1** was significantly more active against all tested virus strains compared to saponin **2**, which features EchA as the aglycone, suggesting that structural differences in triterpenoids may influence binding affinity or biological activity. Surprisingly, despite Lewis-X being widely regarded as a poor ligand for L-SIGN,[7] triterpenoid saponins **1** and **2** displayed similar inhibitory activity against cells expressing either DC-SIGN or L-SIGN. This unexpected finding may be attributed to the unique presentation of the Lewis-X epitope within the context of the triterpenoid scaffold, which could enhance its interaction with L-SIGN through conformational effects. Alternatively, the hydrophobic triterpenoid core might contribute to nonspecific interactions or stabilize binding to L-SIGN. Further studies are however needed to elucidate the precise molecular mechanisms underlying this dual inhibition. Half maximal inhibitory concentrations (IC_{50}) were determined for active saponins **1** and **2** (Table 2). Remarkably, these compounds emerged as among the most potent monovalent inhibitors of DC-SIGN- and L-SIGN-mediated HIV-1 infection reported to date,[13] with IC_{50} values ranging from 21 to 50 μM. The potent inhibition of DC-SIGN- and L-SIGN-mediated HIV-1 *trans*-infection by saponins **1** and **2** highlights their promise as antiviral agents and underscores the potential of the Lewis-X-containing triterpenoid saponins as a versatile scaffold for further optimization.

3. Conclusions

This study reports the first synthesis of Lewis-X-containing BetA and EchA triterpenoid saponins **1** and **2**. A convergent glycosylation approach was employed, utilizing a universal Lewis-X TCA donor that was stereoselectively and efficiently coupled at the C3 position of the parent triterpenoids. Saponins **1** and **2** were obtained in overall yields of 42 and 25%, respectively, over six linear steps from corresponding allyl-protected triterpenoids. In our first parallel work, we synthesized triterpenoid saponins **3** and **4** bearing a trisaccharide with rhamnose replacing fucose for comparative analysis. Notably, Lewis-X-containing triterpenoid saponins **1** and **2** emerged as potent monovalent inhibitors of DC-SIGN- and L-SIGN-mediated HIV-1 transfer to CD4-positive cells, with IC_{50} values ranging from 21 to 50 μM. Our findings suggest that these saponins hold promise for preventing early stages of HIV-1 infection while being relatively nontoxic in vivo, as revealed in our second parallel work. Furthermore, as DC-SIGN and L-SIGN facilitate infection by various microbial pathogens,

we are currently investigating the inhibitory activity of saponins **1** and **2** against other pathogens. Future studies will also explore binding kinetics to provide a deeper mechanistic understanding of the inhibitory activities observed. Additionally, these saponins could be formulated into liposomes or dendrimeric structures to reach multivalent presentation of Lewis-X epitopes. Efforts to achieve these aims are currently underway in our research group.

Supporting Information

Synthetic and infection assay procedures, experimental data, additional figures and schemes, and NMR spectra for all new compounds. This material is available free of charge via the Internet. The authors have cited additional references within the Supporting Information.[53]

Author Contributions

The manuscript was written with contributions from all authors. All authors have approved the final version of the manuscript. Conceptualization (O. J. G. M. and C. G.), formal analysis (O. J. G. M., K. N., R. P., and C. G.), funding acquisition (R. P. and C. G.), investigation (O. J. G. M., K. N., N. V., and A. G. F. Y.), project administration (R. P. and C. G.), supervision (R. P. and C. G.), writing–original draft preparation (O. J. G. M. and C. G.), writing–review and editing (O. J. G. M., K. N., N. V., R. P., and C. G.).

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Conflict of Interests

The authors declare no competing financial interests.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

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[1] a) O. O. Oguntibeju, *HIV AIDS* **2012**, *4*, 117; b) T. C. Quinn, *AIDS* **2008**, *22*, S7.
[2] a) L. Bailon, B. Mothe, L. Berman, C. Brander, *Drugs* **2020**, *80*, 859; b) J. Popović-Djordjević, C. Quispe, R. Giordo, A. Kostić, J. S. Katanić Stanković, P. V. Tsouh Fokou, K. Carbone, M. Martorell, M. Kumar, G. Pintus, J. Sharifi-Rad, A. O. Docea, D. Calina, *Eur. J. Med. Chem.* **2022**, *233*, 114217.
[3] J. Banchereau, F. Briere, C. Caux, J. Davoust, S. Lebecque, Y.-J. Liu, B. Pulendran, K. Palucka, *Ann. Rev. Immunol.* **2000**, *18*, 767.
[4] C. G. Figdor, G. J. Adema, *Nat. Rev. Immunol.* **2002**, *2*, 77.
[5] S. J. van Vliet, J. J. García-Vallejo, Y. van Kooyk, *Immunol. Cell. Biol.* **2008**, *86*, 580.
[6] a) P. Y. Lozach, L. Burleigh, I. Staropoli, A. Amara, *Methods Mol. Biol.* **2007**, *379*, 51; b) U. S. Khoo, K. Y. Chan, V. S. Chan, C. L. Lin, *J. Mol. Med.* **2008**, *86*, 861.
[7] E. Van Liempt, A. Imberty, C. M. C. Bank, S. J. Van Vliet, Y. Van Kooyk, T. B. H. Geijtenbeek, I. Van Die, *J. Biol. Chem.* **2004**, *279*, 33161.
[8] a) Y. Guo, H. Feinberg, E. Conroy, D. A. Mitchell, R. Alvarez, O. Blixt, M. E. Taylor, W. I. Weis, K. Drickamer, *Nat. Struct. Mol. Biol.* **2004**, *11*, 591; b) B. J. Appelmelk, I. van Die, S. J. van Vliet, C. M. J. E. Vandenbroucke-Grauls, T. B. H. Geijtenbeek, Y. van Kooyk, *J. Immunol.* **2003**, *170*, 1635; c) E. van Liempt, C. Bank, P. Mehta, J. Garciá-Vallejo, Z. Kawar, R. Geyer, R. Alvarez, R. Cummings, Y. van Kooyk, I. van Die, *FEBS Lett.* **2006**, *580*, 6123; d) H. Feinberg, D. A. Mitchell, K. Drickamer, W. I. Weis, *Science* **2001**, *294*, 2163.
[9] Y. van Kooyk, T. B. H. Geijtenbeek, *Nat. Rev. Immunol.* **2003**, *3*, 697.
[10] P. W. Hong, K. B. Flummerfelt, A. de Parseval, K. Gurney, J. H. Elder, B. Lee, *J. Virol.* **2002**, *76*, 12855.
[11] a) T. B. H. Geijtenbeek, D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. F. van Duijnhoven, J. Middel, I. L. M. H. A. Cornelissen, H. S. L. M. Nottet, V. N. KewalRamani, D. R. Littman, C. G. Figdor, Y. van Kooyk, *Cell* **2000**, *100*, 587; b) S. Pöhlmann, F. Baribaud, B. Lee, G. J. Leslie, M. D. Sanchez, K. Hiebenthal-Millow, J. Münch, F. Kirchhoff, R. W. Doms, *J. Virol.* **2001**, *75*, 4664.
[12] S. Sattin, F. Fieschi, A. Bernardi, in *Carbohydr. Chem.* **2015**, pp. 379.
[13] M. Anderluh, G. Jug, U. Svajger, N. Obermajer, *Curr. Med. Chem.* **2012**, *19*, 992.
[14] a) G. Timpano, G. Tabarani, M. Anderluh, D. Invernizzi, F. Vasile, D. Potenza, P. M. Nieto, J. Rojo, F. Fieschi, A. Bernardi, *ChemBioChem* **2008**, *9*, 1921; b) M. Andreini, D. Doknic, I. Sutkeviciute, J. J. Reina, J. Duan, E. Chabrol, M. Thepaut, E. Moroni, F. Doro, L. Belvisi, *Org. Biomol. Chem.* **2011**, *9*, 5778; c) J. J. Reina, S. Sattin, D. Invernizzi, S. Mari, L.

Martínez-Prats, G. Tabarani, F. Fieschi, R. Delgado, P. M. Nieto, J. Rojo, A. Bernardi, *ChemMedChem* **2007**, *2*, 1030; d) N. Obermajer, S. Sattin, C. Colombow, M. Bruno, U. Švajger, M. Anderluh, A. Bernardi, *Mol. Divers.* **2010**, *15*, 347; e) S. Sattin, A. Daghetti, M. Thépaut, A. Berzi, M. Sánchez-Navarro, G. Tabarani, J. Rojo, F. Fieschi, M. Clerici, A. Bernardi, *ACS Chem. Biol.* **2010**, *5*, 301; f) T. Tomašić, D. Hajšek, U. Švajger, J. Luzar, N. Obermajer, I. Petit-Haertlein, F. Fieschi, M. Anderluh, *Eur. J. Med. Chem.* **2014**, *75*, 308.

[15] a) N. Varga, I. Sutkeviciute, R. Ribeiro-Viana, A. Berzi, R. Ramdasi, A. Daghetti, G. Vettoretti, A. Amara, M. Clerici, J. Rojo, F. Fieschi, A. Bernardi, *Biomaterials* **2014**, *35*, 4175; b) S. Ordanini, N. Varga, V. Porkolab, M. Thépaut, L. Belvisi, A. Bertaglia, A. Palmioli, A. Berzi, D. Trabattoni, M. Clerici, F. Fieschi, A. Bernardi, *Chem. Commun.* **2015**, *51*, 3816; c) J. J. Garcia-Vallejo, N. Koning, M. Ambrosini, H. Kalay, I. Vuist, R. Sarrami-Forooshani, T. B. H. Geijtenbeek, Y. van Kooyk, *Int. Immunol.* **2013**, *25*, 221; d) O. Martínez-Avila, K. Hijazi, M. Marradi, C. Clavel, C. Campion, C. Kelly, S. Penadés, *Chem. Eur. J.* **2009**, *15*, 9874; e) A. Muñoz, D. Sigwalt, B. M. Illescas, J. Luczkowiak, L. Rodríguez-Pérez, I. Nierengarten, M. Holler, J.-S. Remy, K. Buffet, S. P. Vincent, J. Rojo, R. Delgado, J.-F. Nierengarten, N. Martín, *Nat. Chem.* **2016**, *8*, 50; f) R. Ribeiro-Viana, M. Sánchez-Navarro, J. Luczkowiak, J. R. Koeppe, R. Delgado, J. Rojo, B. G. Davis, *Nat. Commun.* **2012**, *3*, 1303.

[16] a) H. Zhang, O. Daněk, D. Makarov, S. Rádl, D. Kim, J. Ledvinka, K. Vychodilová, J. Hlaváč, J. Lefèbre, M. Denis, C. Rademacher, P. Ménová, *ACS Med. Chem. Lett.* **2022**, *13*, 935; b) M. J. Borrok, L. L. Kiessling, *J. Am. Chem. Soc.* **2007**, *129*, 12780; c) S. L. Mangold, L. R. Prost, L. L. Kiessling, *Chem. Sci.* **2012**, *3*, 772; d) K. C. A. Garber, K. Wangkanont, E. E. Carlson, L. L. Kiessling, *Chem. Commun.* **2010**, *46*, 6747; e) L. R. Prost, J. C. Grim, M. Tonelli, L. L. Kiessling, *ACS Chem. Biol.* **2012**, *7*, 1603.

[17] L. de Witte, A. Nabatov, M. Pion, D. Fluitsma, M. A. W. P. de Jong, T. de Gruijl, V. Piguet, Y. van Kooyk, T. B. H. Geijtenbeek, *Nat. Med.* **2007**, *13*, 367.

[18] a) M. Naarding, I. Ludwig, F. Groot, B. Berkhout, T. Geijtenbeek, G. Pollakis, W. Paxton, *J. Clin. Invest.* **2005**, *115*, 3256; b) P. Hong, M. Ninonuevo, B. Lee, C. Lebrilla, L. Bode, *Br. J. Nutr.* **2009**, *101*, 482.

[19] V. Zuco, R. Supino, S. C. Righetti, L. Cleris, E. Marchesi, C. Gambacorti-Passerini, F. Formelli, *Cancer Lett.* **2002**, *175*, 17.

[20] a) S. C. Jonnalagadda, P. Suman, D. C. Morgan, J. N. Seay, in *Studies in Natural Products Chemistry*, Vol. *53*, (Ed.: R. Atta ur), Elsevier, **2017**, pp. 45–84; b) P. A. Krasutsky, *Nat. Prod. Rep.* **2006**, *23*, 919.

[21] C. Aiken, C. H. Chen, *Trends Mol. Med.* **2005**, *11*, 31.

[22] K. Qian, T. J. Nitz, D. Yu, G. P. Allaway, S. L. Morris-Natschke, K.-H. Lee, in *Natural Product Chemistry for Drug Discovery* (Eds.: A. D. Buss, M. S. Butler), The Royal Society of Chemistry, **2009**, pp. 374–391.

[23] M. W. C. Loe, E. Hao, M. Chen, C. Li, R. C. H. Lee, I. X. Y. Zhu, Z. Y. Teo, W. X. Chin, X. Hou, J. Deng, J. J. H. Chu, *Antiviral Res.* **2020**, *184*, 104954.

[24] X. Han, Y. Shi, L. Si, Z. Fan, H. Wang, R. Xu, P. Jiao, K. Meng, Z. Tian, X. Zhou, H. Jin, X. Wu, H. Chen, Y. Zhang, L. Zhang, S. Xiao, D. Zhou, *MedChemComm* **2016**, *7*, 1932.

[25] M. Liu, J. Wang, S. Shi, Y. Gao, Y. Zhang, Z. Yuan, E. Huang, S. Li, S. Liu, G. Song, *Eur. J. Med. Chem.* **2023**, *256*, 115463.

[26] a) K. Muru, C. Gauthier, *Chem. Rec.* **2021**, *11*, 2990; b) C. Gauthier, J. Legault, M. Piochon-Gauthier, A. Pichette, *Phytochem. Rev.* **2011**, *10*, 521.

[27] C. Gauthier, J. Legault, K. Girard-Lalancette, V. Mshvildadze, A. Pichette, *Bioorg. Med. Chem.* **2009**, *17*, 2002.

[28] a) F. Yu, Q. Wang, Z. Zhang, Y. Peng, Y. Qiu, Y. Shi, Y. Zheng, S. Xiao, H. Wang, X. Huang, L. Zhu, K. Chen, C. Zhao, C. Zhang, M. Yu, D. Sun, L. Zhang, D. Zhou, *J. Med. Chem.* **2013**, *56*, 4300; b) H. Wang, F. Yu, Y. Peng, Q. Wang, X. Han, R. Xu, X. Zhou, C. Wan, Z. Fan, P. Jiao, Y. Zhang, L. Zhang, D. Zhou, S. Xiao, *Eur. J. Med. Chem.* **2015**, *102*, 594.

[29] A. Fernandez-Tejada, E. K. Chea, C. George, N. Pillarsetty, J. R. Gardner, P. O. Livingston, G. Ragupathi, J. S. Lewis, D. S. Tan, D. Y. Gin, *Nat. Chem.* **2014**, *6*, 635.

[30] O. J. Gamboa Marin, N. Verma, M. Cloutier, C. Gauthier, *Eur. J. Org. Chem.* **2025**, e202500285.

[31] O. J. Gamboa Marin, Y. Adda-Bouchard, B. Sylla, N. Verma, T. Charpentier, M. Huber, G. Lopez, A. Pichette, A. Lamarre, C. Gauthier, *Chem. Eur. J.* **2025**, e202500994.

[32] J. L. C. Sousa, C. S. R. Freire, A. J. D. Silvestre, A. M. S. Silva, *Molecules* **2019**, *24*, 355.

[33] C. Gauthier, J. Legault, M. Lebrun, P. Dufour, A. Pichette, *Bioorg. Med. Chem.* **2006**, *14*, 6713.

[34] S.-J. Ge, Y.-H. Tu, J.-H. Xia, J.-S. Sun, *Eur. J. Org. Chem.* **2017**, *2017*, 3929.

[35] G. Blatter, J. M. Beau, J. C. Jacquinet, *Carbohydr. Res.* **1994**, *260*, 189.

[36] R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.

[37] B. Yu, J. Xie, S. Deng, Y. Hui, *J. Am. Chem. Soc.* **1999**, *121*, 12196.

[38] M. S. Ehret, M. Virlouvet, M. B. Biskup, *Synthesis* **2010**, *2010*, 3481.

[39] J. Marz, H. Kunz, *Synlett* **1992**, 589.

[40] R. U. Lemieux, K. B. Hendriks, R. V. Stick, K. James, *J. Am. Chem. Soc.* **1975**, *97*, 4056.

[41] a) C. Gauthier, J. Legault, M. Piochon, S. Lavoie, S. Tremblay, A. Pichette, *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2310; b) J. A. R. Salvador, R. M. A. Pinto, R. C. Santos, C. Le Roux, A. M. Beja, J. A. Paixao, *Org. Biomol. Chem.* **2009**, *7*, 508.

[42] S. Rio, J. M. Beau, J. C. Jacquinet, *Carbohydr. Res.* **1991**, *219*, 71.

[43] Z. Gan, S. Cao, Q. Wu, R. Roy, *J. Carbohydr. Chem.* **1999**, *18*, 755.

[44] A. Wang, J. Hendel, F.-l. Auzanneau, *Beilstein J. Org. Chem.* **2010**, *6*, 17.

[45] a) D. Baudry, M. Ephritikhine, H. Felkin, *J. Chem. Soc. Chem. Commun.* **1978**, 694; b) J. J. Oltvoort, C. A. A. Van Boeckel, J. H. De Koning, J. H. Van Boom, *Synthesis* **1981**, 305.

[46] M. Mende, C. Bednarek, M. Wawryszyn, P. Sauter, M. B. Biskup, U. Schepers, S. Bräse, *Chem. Rev.* **2016**, *116*, 8193.

[47] A. Toepfer, R. R. Schmidt, *J. Carbohydr. Chem.* **1993**, *12*, 809.

[48] a) N. Barroca-Aubry, A. Pernet-Poil-Chevrier, A. Domard, S. Trombotto, *Carbohydr. Res.* **2010**, *345*, 1685; b) E. R. Palmacci, P. H. Seeberger, *Tetrahedron* **2004**, *60*, 7755.

[49] T. Hideki, A. Hiromune, T. Hide-Nori, H.-Y. Ritsuko, Y. Tomio, I. Hideharu, K. Makoto, *Angew. Chem., Int. Ed.* **2011**, *50*, 2330.

[50] T. J. Donohoe, J. G. Logan, D. D. P. Laffan, *Org. Lett.* **2003**, *5*, 4995.

[51] I. Tvaroska, F. R. Taravel, *Adv. Carbohydr. Chem. Biochem.* **1995**, *51*, 15.

[52] F. Guibé, *Tetrahedron* **1998**, *54*, 2967.

[53] a) S. U. Hansen, G. J. Miller, M. Baráth, K. R. Broberg, E. Avizienyte, M. Helliwell, J. Raftery, G. C. Jayson, J. M. Gardiner, *J. Org. Chem.* **2012**, *77*, 7823; b) J. Boutet, C. Guerreiro, L. A. Mulard, *Tetrahedron* **2008**, *64*, 10558; c) B. N. A. Mbadugha, F. M. Menger, *Org. Lett.* **2003**, *5*, 4041.

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